Universidade Estadual de Londrina

LUIZ DANIEL DE BARROS

AVALIAÇÃO DOS POMBOS DA ESPÉCIE *ZENAIDA AURICULATA* COMO HOSPEDEIRO INTERMEDIÁRIO DO *NEOSPORA CANINUM*

Londrina
2016

LUIZ DANIEL DE BARROS

AVALIAÇÃO DOS POMBOS DA ESPÉCIE *ZENAIDA AURICULATA* COMO HOSPEDEIRO INTERMEDIÁRIO DO *NEOSPORA CANINUM*

Tese de doutorado apresentada ao Programa de Pós-graduação em Ciência Animal (áre de concentração: Sanidade Animal) do Centro de Ciências Agrárias da Universidade Estadual de Londrina, como requisito para à obtenção do título de Doutor em Ciência Animal

Orientador: Prof. Dr. João Luis Garcia

Londrina
2016

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

de Barros, Luiz Daniel.
Avaliação dos pombos da espécie Zenaida auriculata como hospedeiro intermediário do Neospora caninum / Luiz Daniel de Barros. - Londrina, 2016.
80 f. : il.

Orientador: João Luis Garcia.
Tese (Doutorado em Ciência Animal) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência Animal, 2016.
Inclui bibliografia.

1. Neosporose - Teses. 2. Pombo - Teses. 3. Sorologia - Teses. 4. ELISA - Teses. I. Garcia, João Luis. II. Universidade Estadual de Londrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência Animal. III. Título.

LUIZ DANIEL DE BARROS

AVALIAÇÃO DOS POMBOS DA ESPÉCIE *ZENAIDA AURICULATA* COMO HOSPEDEIRO INTERMEDIÁRIO DO *NEOSPORA CANINUM*

Tese de doutorado apresentada ao Programa de Pós-graduação em Ciência Animal (área de concentração: Sanidade Animal) do Centro de Ciências Agrárias da Universidade Estadual de Londrina, como requisito para obtenção do título de Doutor em Ciência Animal.

BANCA EXAMINADORA

____
Orientador: Prof. Dr. João Luis Garcia
Universidade Estadual de Londrina – UEL

____
Prof. Dr. Odilon Vidotto
Universidade Estadual de Londrina – UEL

____
Prof. Dr. José da Silva Guimarães Junior
Universidade Estadual de Londrina – UEL

____
Prof. Dr. Anderson Barbosa de Moura
Universidade do Estado de Santa Catarina – UDESC

____
Prof. Dr. Alexey Leon Gomel Bogado
Universidade Norte do Paraná – UNOPAR

Londrina, 01 de abril de 2016.

AGRADECIMENTOS

Agradeço à Deus, por estar sempre iluminando os meus caminhos à serem percorridos.

Aos meus pais, Luiz Carlos de Barros e Marisa Sávio de Barros, e aos meus irmãos, Ana Márcia, Luiz Guilherme e Luiz Miguel, por todo o amor, carinho e incentivo em minha vida, apesar dos anos de distância.

Ao meu orientador, Prof. Dr. João Luis Garcia, pela orientação, confiança, ensinamentos e sobretudo pela amizade ao longo desses anos.

Ao professor Dr. Odilon Vidotto, pela colaboração no trabalho e ajuda nos momentos em que sempre precisei.

Ao professor Dr. Chunlei Su, pela recepção e ensinamentos no período do estágio no exterior.

Ao professor Dr. Selwyn Arlington Headley, pelas contribuições e ajuda no trabalho.

Aos professores Dr. Anderson Barbbosa de Moura, Dr. José da Silva Guimarães Junior e Dr. Alexey Leon Gomel Bogado pela participação na banca da defesa.

Aos professores Dr. Italmar Teodorico Navarro, Dra. Regina Mitsuka-Breganó e Dra. Roberta Lemos Freire pelos ensinamentos e ajuda ao longo desses anos de pós-graduação

À companheira de projeto Alessandra Taroda, pela ajuda e convívio nos trabalhos com os pombos.

Aos amigos que fiz durante essa jornada, José Mauricio Ferreira Neto, Jonatas Campos de Almeida, Dauton Luiz Zulpo, Ivo Alexandre Leme da Cunha e Fernanda Evers pela amizade e momentos de diversão fora do laboratório.

Aos amigos de pós-graduação Sérgio Tosi Cardim, Ana Carolina Miura, Ana Sue Sammi, Mércia de Seixas, Felippe Martins, Ana Flávia Minutti, Nelson dos Santos, Vanessa Holsbach, Thais Agostinho, Thais Cabral, João Pedro Sasse, Andressa Rorato, Fernanda Ferreira, Marcelle Mareze, Hugo Lucca, Ricardo Matos pelo convívio no dia a dia do laboratório.

À amiga de pós-graduação Elisângela Olegário da Silva, pela amizade durante todos esses anos e especial ajuda com as análises histopatológicas.

Aos estagiários do laboratório Isabelli Kono, Winni Ladeia, Lya Bordini, Caroline Ambiel e Alisson Marques pela colaboração em várias etapas do projeto.

À estagiária Camila Minuzzi, da UFSM, por toda ajuda no experimento, especialmente com os cães durante o período de estágio curricular.

Ao residente Henrique Vilella e toda a equida de cirurgia do Departamento de Clínicas Veterinária da UEL pelos procedimentos cirúrgicos de castração dos cães.

Aos amigos que fiz nos Estados Unidos, especialmente às brasileiras Flávia Pereira Vieira, Priscila Silva Franco e Bruna Yassuhara pelas aventuras e ajuda na terra do tio Sam.

Aos técnicos de laboratório Beatriz Nino, Elizabete Marana, Dalva Navarro e Aldair Matos pela ajuda nas tarefas diárias do laboratório.

Aos secretários Helenice e Valdecir, pela prontidão nos momentos em que precisei.

Aos animais utilizados no projetos, pela contribuição nas pesquisas e avanço da ciência.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES pela concessão da bolsa de doutorado

À todos que contribuiram direta ou indiretamento neste trabalho, pois a ajuda de vocês foi imprescindível para a realização desse trabalho.

You can’t climb the ladder of success with
your hands in your pockets
(Arnold Schwarzenegger)

BARROS, Luiz Daniel de . **Avaliação dos pombos da espécie *Zenaida* auriculata como hospedeiro intermediário do *Neospora caninum***. 2016. 80f. Tese de doutorado (Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2016.

RESUMO

Neospora caninum é um protozoário, parasita intracelular obrigatório pertencente filo Apicomplexa e família Sarcocystidae. O parasita possui distribuição mundial e é responsável pela neosporose, uma doença primária de cães e bovinos, sendo considerada como uma das principais causas de abortamento em bovinos. Cães e outros canídeos são os hospedeiros definitivos e eliminam oocistos no ambiente. Vários animais domésticos e silvestres, incluindo aves, tem sidos descritos como hospedeiros intermediários, entretanto o papel desses animais no ciclo epidemiológico ainda é incerto. O objetivo do presente estudo foi avaliar se pombos da espécie *Zenaida auriculata* podem atuar como hospedeiros intermediários do *N. caninum*, contribuindo assim com a transmissão da doença. No primeiro experimento, 249 pombos *Z. auriculata* foram capturados em três diferentes áreas da cidade de Londrina, Paraná. Amostras de soro e cérebro foram coletados para a realização dos testes sorológicos e molecular respectivamente. Por meio da técnica de ELISA, 31,72% (79/249) das amostras foram positivas, com maior número de aves positivas capturadas em uma indústria de processamento de grãos. Não foi observado DNA do parasita utilizando a técnica de PCR em nenhuma das amostras de tecido cerebral. No segundo experimento, 18 pombos foram divididos em 4 grupos, G1, G2 e G3, composto por 5 animais cada, receberam $2x10_6$ taquizoítos da cepa NC-1 do *N. caninum* pelas vias subcutânea, intramuscular e intraperitoneal respectivamene. G4, composto por 3 animais, permaneceu como grupo controle. A técnica de ELISA foi utilizada para verificar a cinética dos anticorpos contra o parasita após a inoculação. No sétimo e 14° dia, um animal dos grupos G1, G2 e G3 foram eutanasiados para detecção do parasita pela PCR. Após 28 dias, todos os outros animais foram eutanasiados e tecidos (cérebro, coração, fígado, pulmão, rim e músculo peitoral) foram coletados para análise de PCR e exame histopatológico. Foi realizado bioensaio em cães para verificar cistos na musculatura de pombos experimentalmente infectados. Quatro cães foram foram alimentados com tecidos de pombos inoculados previamente de somente um grupo. Exames coproparasitológico e imunológico foram realizados para avaliar a infecção nos cães. Todas as aves inoculadas soroconverteram após desafio, com maior produção de anticorpos nas aves inoculadas pela via intraperitoneal. Um animal do grupo 3 eutanaziado no 7 dia após inoculação foi positivo na PCR dos tecidos. Apenas um animal do G3 apresentou um infiltrado inflamatório mononuclear no pulmão e rim, compatível com neosporose no exame histopatológico. Nenhum cão soroconverteu ou eliminou oocistos durante o período experimental. Esse estudo verificou pela primeira vez a presença de anticorpos contra *N. caninum* em pombos *Z. auriculata* de vida livre, demonstrando que esses animais tiveram um contato prévio com o *N. caninum*. Nossos resultados também indicaram que a via intraperitoneal induziu infecção nos pombos, entretanto essas aves foram consideradas resistente à infecçςao crônica. Estudos adicionais devem ser conduzidos, especialmente utilizando outros modelos de infecção, para avaliar a importância dos pombos *Z. auriculata* na epidemiologia da neosporose.

Palavras-chave: Neosporose. Pombo. Sorologia. ELISA. Bioensaio em cães.

BARROS, Luiz Daniel. **Evaluation of doves from *Zenaida auriculata* species as intermediate host of *Neospora caninum***. 2016. 80p. Thesis (Animal Science) – Universidade Estadual de Londrina, Londrina, 2016.

ABSTRACT

Neospora caninum is a protozoan, obligate intracelular parasite that belongs to Apicomplexa phylum and Sarcocystidae family. The parasite has worlwide distribution and is resposible for neosporosis, a primarily in dogs and cattle, being considered as one of the major causes of abortion in cattle. Dogs ans other canids are the definitve hosts e shed oocysts in the enviroment. Many domestic and wild animals, including birds, have been described as intermediate host, however, the role of these animals in epidemiological cycle is uncertain. The aim of the present study was to evaluate if doves from *Zenaida auriculata* species can act as intermediate host of *N. caninum*, contributing with transmission of the disease. In the first experiment, 249 dove *Z. auriculata* were captured in three different areas from Londrina city, Paraná. Serum and brain samples were collected to perform serological and molecular tests respectively. By means of ELISA, 31.72% (79/249) of samples were positive, with higher number of positive birds captured in an soybean seed plant. It was not observed DNA from the parasite using the PCR technique in all brain tissue samples tested. In the second experiment, 18 doves were divided in 4 groups, G1, G2 and G3, composed by 5 animals each, received $2x10^6$ tachyzoites from NC-1 strain of *N. caninum* by subcutaneous, intramuscular and intraperitoneal route respectively. G4, composed by 3 animals, remained as control group. ELISA technique was used to verify the kinetics of antibodies against the parasite after inoculation. On 7th and 14th day, one animal from G1, G2 and G3 groups, were euthanized to detect the parasite by PCR. After 28 days, all other animals were euthanized and tissue (brain, heart, liver, lung, kidney and pectoral muscle) were collected for PCR analysis and histopathological examination. Bioassay in dogs were performed to verify cysts in the musculature of doves infected experimentally. Four dogs were fed with doves tissue from only one group. Coproparasitological and immunological examination were performed to evaluate dogs infection. All birds inoculated seroconverted after challenge, with higher antibodies production in birds inoculated through intraperitoneal route. One animal from group 3 that was euthanized on 7th day after inoculation was positive in PCR from tissues. Only one animal from G3 showed an mononuclear inflammatory infiltrate in lung and kidney, which is compatible with neosporosis in the histopathology evaluation. None dogs seroconverted or shed oocysts during experimental period. This study verified for the first time the presence of antibodies against *N. caninum* in free-ranging doves *Z. auriculata*, showing that these animals had previously contact with *N. caninum*. Our results also indicated that intraperitoneal route induced infection in doves, however these birs were considered resistent to chronic infection. Aditional studies should be conducted, especially using other infection models, to evaluate the importance of *Z. auriculata* doves in the epidemiology of neosporosis.

Keywords: Neosporosis. Eared doves. Serology. ELISA. Dogs bioassay.

LISTA DE ILUSTRAÇÕES

REFERENCIAL TEÓRICO

Figura 1 – Formas parasitárias do *Neospora caninum.* A) Cisto tecidual 30 com bradizoítos no interior. B) Taquizoítos corados com Giemsa. C) Oocisto 31 esporulado. 12

Figura 2 – Ciclo Biológico do *Neospora caninum*..13

SURVEY OF NEOSPORA CANINUM IN EARED DOVES (ZENAIDA AURICULATA) FROM BRAZIL

Figura 1 – Serological result by an ELISA test for *N. caninum* in eared doves (*Zenaida auriculata*) trap-captured from different location in Southern Brazil. Dashed line indicates the positive cut off (0.433) and line the mean..59

EXPERIMENTAL INOCULATION OF NEOSPORA CANINUM TACHYZOITES IN EARED DOVES (ZENAIDA AURICULATA)

Figura 1 – Immunological response of eared doves (*Zenaida auriculata*) inoculated with *Neospora caninum* tachyzoites evaluated by the indirect enzyme-linked immunosorbent assay (ELISA). All birds received the same parasite concentration by different routes, being G1 subcutaneous, G2 intramuscular and G3 intraperitoneal route (bars= OD mean from animals and error bars= standard deviation). Dashed line indicates positive cut off (OD=0.4579) 74

LISTA DE TABELAS

REFERENCIAL TEÓRICO

Tabela 1 – Serological prevalence of *Neospora caninum* in birds from different countries. 38

Tabela 2 – Birds as natural intermediate hosts for *Neospora caninum* due to detection of the parasite using different methods from bird tissues 40

Tabela 3 – Mammals as natural intermediate hosts for *Neospora caninum* due to detection of the parasite using different methods from animal tissues 41

SURVEY OF NEOSPORA CANINUM IN EARED DOVES (ZENAIDA AURICULATA) FROM BRAZIL

Tabela 1 – Association of sex and capture site with the presence of antibodies against *N. caninum* by an ELISA test in eared doves (*Zenaida auriculata*) from Southern Brazil 58

EXPERIMENTAL INOCULATION OF NEOSPORA CANINUM TACHYZOITES IN EARED DOVES (ZENAIDA AURICULATA)

Tabela 1 – Detection of *Neospora caninum* by different test in eared doves (*Zenaida auriculata*) inoculated with tachyzoites of NC-1strain. 73

SUMÁRIO

1 INTRODUÇÃO 08

2 REFERENCIAL TEÓRICO 10

REFERÊNCIAS 16

3 ARTIGO DE REVISÃO PARA PUBLICAÇÃO 21

1 INTRODUCTION 22

2 Serological studies 24

3 Experimental infections 26

4 Natural infections 28

5 Conclusions 32

References 32

4 OBJETIVOS 43

4.1 OBJETIVO GERAL 43

4.2 OBJETIVOS ESPECÍFICOS 43

5 ARTIGO PARA PUBLICAÇÃO I 44

Abstract 45

Introduction 45

2 Material and methods 46

2.1 Animals and study locations 46

2.2 Sample collection 47

2.3 Serology 47

2.4 DNA extraction and PCR 49

2.5 Statistical analysis 49

3 Results 50

4 Discussion 50

5 Conclusions 53

References 53

6 ARTIGO PARA PUBLICAÇÃO II 60

Abstract 61

1 Introduction 61
2 Material and methods..62
2.1 Animals 63
2.2 *Neospora caninum* strain 63
2.3 Experimental design...63
2.4 Sample collection 64
2.5 Enzyme-linked immunosorbent assay test (ELISA) 64
2.6 DNA extraction and PCR..65
2.7 Histopathology 66
2.9 Bioassay in dogs 66
2.9 Indirect fluorescent antibody test (IFAT)..................................66
2.10 Coproparasitological examinations 67
3 Results 67
4 Discussion...68
5 Conclusions...69
Reference 69

CONCLUSÃO 75

ANEXOS 76
Anexo A - Mapa do local de captura dos pombos: Campus universitário; B:Indústria de processamento de grãos; C: Fazenda de bovinocultura leiteira. 77
Anexo B – Ofício do Comitê de Ética em Experimentação Animal n° 70/2008 78
Anexo C – Ofício da Comissão de Ética no Uso de Animais n° 157/2015 79
Anexo D - Autorização do Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA n° 16428-1 80

1 INTRODUÇÃO

A neosporose é uma doença infecciosa de origem parasitária causada pelo protozoário *Neospora caninum* (DUBEY et al., 1988). A doença é de grande importância na bovinocultura mundial, devido aos problemas reprodutivos ocasionados (DUBEY; SCHARES, 2006). O cão doméstico e outros membros da família Canidae são os hospedeiros definitivos do parasita, sendo responsáveis pela eliminação de oocistos no ambiente (MCALLISTER et al., 1998; DUBEY et al., 2011). Exames moleculares, histopatológicos e bioensaio são ferramentas amplamente utilizadas para a identificação dos hospedeiros intermediários, sendo atualmente reconhecido mais de 30 espécies de mamíferos e poucas espécies de aves que atuam como hospedeiro intermediário do *N. caninum* (DUBEY; SCHARES, 2011; DONAHOE et al., 2015).

A infecção nos animais pode ocorrer via vertical, forma mais comum nos bovinos, ou por via horizontal por meio da ingestão de alimentos, água contaminados com oocistos ou ingestão de tecidos de hospedeiros contendo cistos (DUBEY; SCHARES; ORTEGA-MORA, 2007). As aves se infectam pela ingestão de oocistos esporulados presente no ambiente, entretanto, o real papel das aves no ciclo biológico do *N. caninum* ainda é incerto (DONAHOE et al., 2015).

Pombos da espécie *Zenaida auriculata* (Des Murus, 1847) são aves pertencentes à ordem dos Columbiformes, nativas da América Central e do Sul, sendo observadas das Antilhas até a Terra do Fogo (SOUZA et al., 2007). Popularmente conhecidas por nomes como amargosinha, pomba-de-bando e avoante, sua identificação é feita por meio da observação de características como coloração acinzentada, tamanho intermediário de aproximadamente 21 cm, duas faixas negras horizontais na lateral da cabeça e manchas também negras nas asas (SICK, 2001).

Nos últimos anos, a população dessas aves tem aumentado consideravelmente nos estados do Sul do país, principalmente devido ao hábito alimentar dessas aves, que é composta por grãos como trigo, milho, soja, além de semente de plantas e resíduos alimentares produzidos pelo homem (RANVAUD et al., 2001). Em regiões com alta produção desses grãos, essas aves são consideradas como pragas agrícolas, causando elevados prejuízos econômicos

(BUCHER; RANVAUD, 2006). Essas aves são consideradas animais sinantrópicos devido à relação de proximidade com o homem, o que torna preocupante a transmissão de patógenos, visto que os pombos podem carrear estágios infectantes de várias doenças, além dos danos ao ambiente público devido ao grande acúmulo de fezes nos grandes centros urbanos (DIAZ et al., 2008; SHIBATTA et al., 2009; BARROS et al., 2014).

O fato dos pombos serem presas fáceis para os canídeos aliado ao hábito de alimentação das aves ser realizada principalmente no solo, ficando expostas à infecção por oocistos presentes no ambiente, torna esses animais como potenciais hospedeiros intermediário do *N. caninum*, justificando assim estudos que avaliem a importância epidemiológica dessas aves no ciclo biológico do parasita.

1 **2 REFERENCIAL TEÓRICO**

2

3 *Neospora caninum* é um parasita intracelular obrigatório pertencente
4 ao filo Apicomplexa. Infecta uma ampla gama de hospedeiros e é causador da
5 neosporose, uma importante doença infecciosa causadora de problemas
6 reprodutivos e síndrome neuromuscular em bovinos e cães, respectivamente, além
7 de já ter sido reportada em outros animais domésticos e silvestres (DUBEY;
8 SCHARES; ORTEGA-MORA, 2007).
9 O parasita foi descrito pela primeira vez na Noruega por Bjerkås,
10 Mohn e Presthus (1984) que relataram a presença de cistos de protozoários no
11 sistema nervoso central e muscular em cães da raça Boxer, provenientes da mesma
12 ninhada com sinais neurológicos e paresia. Embora a doença descrita fosse
13 semelhante à toxoplasmose, os autores não detectaram anticorpos contra esse
14 parasita, além de observarem uma incapacidade de infectar camundongos. Anos
15 mais tarde, Bjerkås e Presthus (1988) através de estudos imunohistoquímicos e
16 ultraestruturais, concluiram que os cistos observados nesses cães com sinais
17 neurológicos eram diferentes do *T. gondii* e se tratava de uma nova espécie de
18 parasita. No mesmo ano, Dubey et al. (1988) avaliando cortes histológicos de cães
19 que morreram com doença neurológica semelhante à toxoplasmose concluiram a
20 existência de um novo parasita e o denominaram de *N. caninum*.
21 Após a descrição do parasita até a identificação do seu hospedeiro
22 definitivo, houve um grande intervalo de tempo. Baker et al. (1995) realizaram um
23 estudo experimental com aves carnívoras para avaliar o potencial dessas aves como
24 hospedeiros definitivos do parasita, entretanto os resultados não foram satisfatórios.
25 Somente em 1998, McAllister et al. (1998) descreveram que os cães domésticos
26 (*Canis familiaris*) são hospedeiros definitivos, visto que cães infectados com cistos
27 teciduais eliminaram oocistos não esporulados nas fezes após 8 dias de infecção.
28 Novos estudos foram realizados e mostraram que outros membros da família
29 Canidae, tal qual o coiote (*Canis latrans*) (GONDIM et al., 2004b), o dingo
30 australiano (*Canis lupus dingo*) (KING et al., 2010) e o lobo cinza (*Canis lupus*)
31 (DUBEY et al., 2011) também são hospedeiros definitivos do parasita.
32 A doença tem sido descrita em vários animais domésticos e
33 silvestres, entretanto bovinos apresentam uma maior susceptibilidade à doença,

1 devido aos problemas reprodutivos tais como abortamento, natimortalidade e
2 nascimento de bezerros fracos (DUBEY; SCHARES, 2006, 2011). Estudos
3 demonstram que a neosporose é reponsável por um prejuízo econômico mundial de
4 mais de 1,2 bilhões de dólares ao ano, enquanto no Brasil, essas cifras giram em
5 torno de 153 milhões de dólares, sendo mais de 100 milhões decorrentes da
6 bovinocultura de corte (REICHEL et al., 2013).

7 Atualmente existem vários testes disponíveis para o diagnóstico
8 sorológico da neosporose, como o teste de aglutinação (NAT), a imunofluorescência
9 indireta (RIFI), o ensaio imunoenzimático (ELISA) e o immunoblot (DUBEY;
10 SCHARES, 2011). Entretanto, a RIFI é considerada como prova padrão para o
11 sorodiagnóstico devido à baixa reatividade cruzada com outros parasitas coccídios
12 (BJÖRKMAN; UGGLA, 1999). A presença de anticorpos contra *N. caninum* já foi
13 observada em felinos (MENESES et al., 2014), camelos (SADREBAZZAZ;
14 HADDADZADEH; SHAYAN, 2006), galinhas (MARTINS; KWOK; DUBEY, 2011),
15 equinos (ABREU et al., 2014), lhamas (MORÉ et al., 2008), suínos (FEITOSA et al.,
16 2014), búfalos (REICHEL et al., 2015), ovinos (ROMANELLI et al., 2007), caprinos
17 (TOPAZIO et al., 2014) além dos bovinos (SOUSA et al., 2012) e caninos (ROBBE
18 et al., 2016). Embora a presença de anticorpos anti-*N. caninum* também tenha sido
19 descrita em seres humanos, o potencial zoonótico ainda é incerto, visto que o
20 parasita nunca foi identificado ou isolado de tecido humano (TRANAS; HEINZEN;
21 WEISS, 1999; LOBATO et al., 2006; OSHIRO et al., 2015).

22 Vários animais domésticos já foram descritos como hospedeiros
23 intermediários do parasita, tais quais os bovinos, ovinos e caprinos (DUBEY et al.,
24 1996), roedores, animais silvestres como raposa (NASCIMENTO et al., 2015) e
25 cervídeos (DUBEY; SCHARES, 2011) além de algumas aves, como galinhas
26 (COSTA et al., 2008), pardal (GONDIM et al., 2010) e corvo (SALANT et al., 2015),
27 entretanto, o isolamento do parasita somente foi relatado em bovinos (CAMPERO et
28 al., 2015), búfalos (RODRIGUES et al., 2004), ovinos (KOYAMA et al., 2001), lobo
29 cinza (DUBEY et al., 2014), veado de cauda branca (VIANNA et al., 2005), bisão
30 europeu (BIEŃ; MOSKWA; CABAJ, 2010) e cães (DUBEY et al., 1988).

31 O parasita apresenta três formas biológicas distintas: taquizoítos,
32 bradizoítos e oocistos (Fig.1). Os taquizoítos possuem formato semi-lunar, medem 3-
33 7µm por 1-5µm e são a forma de multiplicação rápida do parasita, sendo encontrado
34 no interior de células como hepatócitos, macrófagos alveolares, miócitos, células

renais, endoteliais vasculares e nervosas (BARR et al., 1991; DUBEY et al., 2002). Os bradizoítos são alongados e delgados, medindo aproximadamente 6,5-8µm por 1,5-2µm e envoltos por um cisto. São a forma lenta de multiplicação do parasita, sendo encontrados principalmente no sistema nervoso central dos hospedeiros intermediários e definitivos. Possuem formato arredondado ou ovalado e variam no tamanho, podendo chegar até 107µm de diâmetro e com parede de até 4µm (DUBEY et al., 2002, 2004). Os oocistos são a forma de resistência do parasita encontrado no ambiente, medem 10,6µm-12,4µm por 10,6µm-12µm e são eliminados nas fezes dos canídeos na forma não esporulada. Após esporulação no meio ambiente, os oocistos são compostos por dois esporocistos, cada qual com quatro esporozoítos, que são alongados e medem 5,8-7,0µm por 1,8-2,2µm (DUBEY et al., 2002).

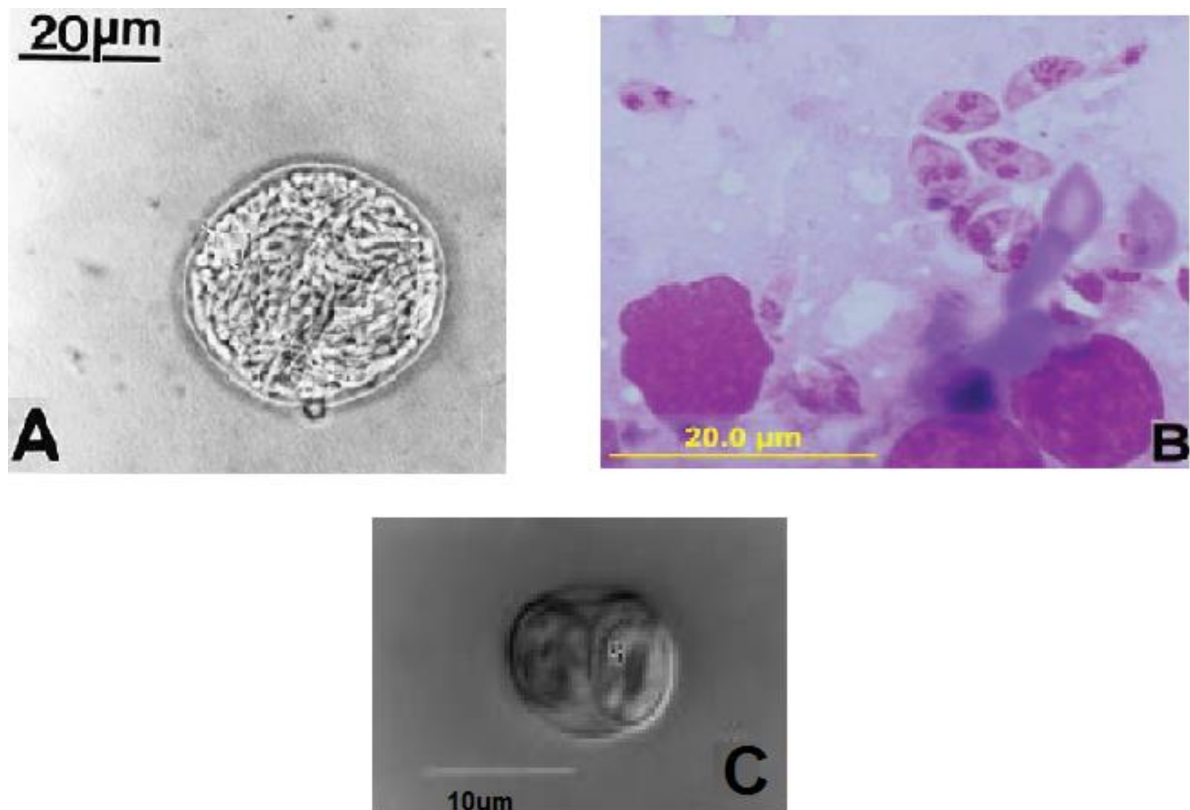
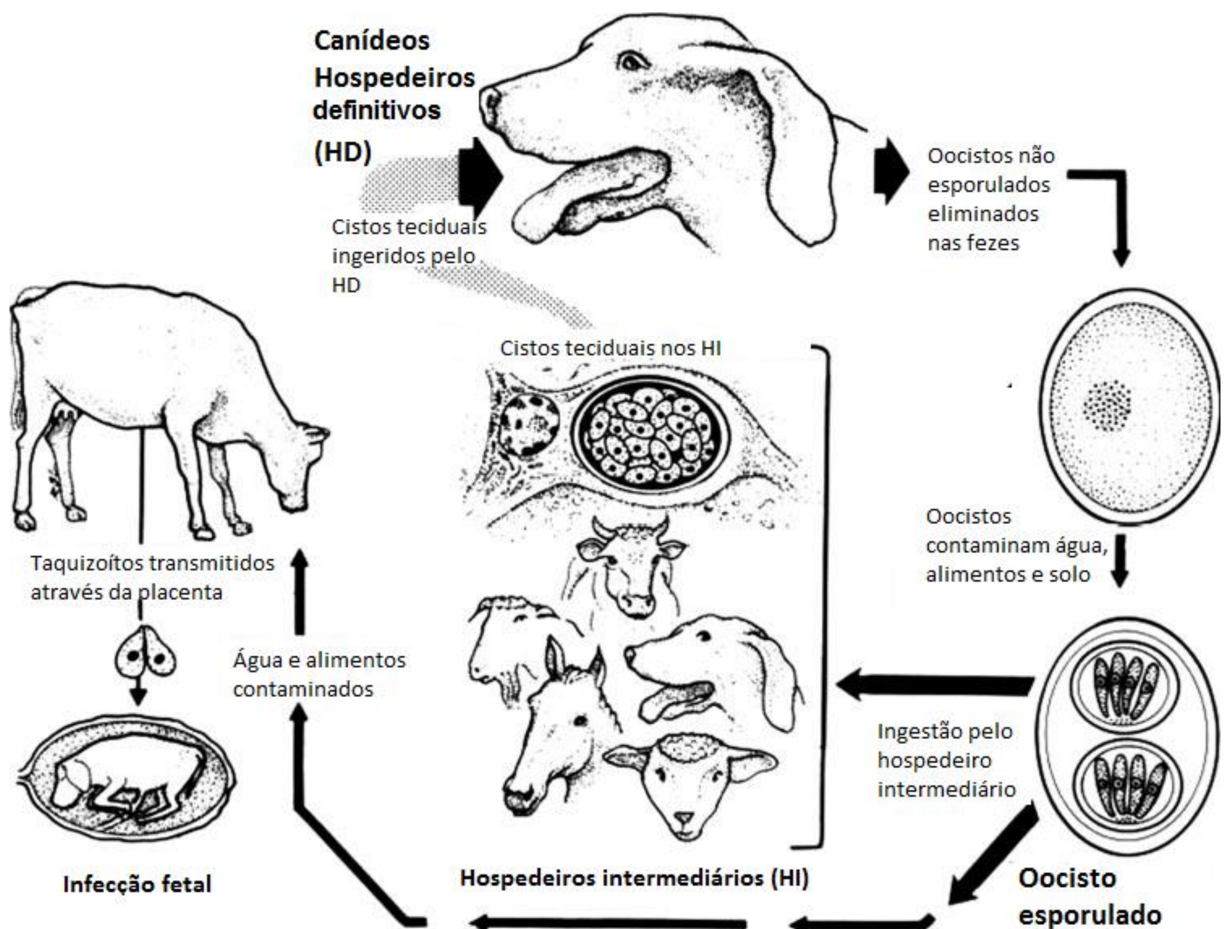


Fig. 1. Formas parasitárias do *Neospora caninum*. A) Cisto tecidual com bradizoítos no interior. B) Taquizoítos corados com Giemsa. C) Oocisto esporulado. Fonte: Adaptado de Lindsay; Upton; Dubey, 1999; Dubey et al., 2004; Dubey; Schares; Ortega-Mora, 2007

1 O ciclo biológico do parasita (Fig. 2) é considerado como
2 heteroxeno facultativo, caracterizado pela presença de um canídeo como hospedeiro
3 definitivo, onde irá ocorrer uma ciclo sexual enteroepitelial, ainda não completamente
4 elucidado, culminando com a eliminação de oocistos nas fezes, e de um hospedeiro
5 intermediário, onde ocorrerá uma fase de multiplicação assexuada, culminando com
6 a formação de cistos teciduais (DUBEY; SCHARES, 2006; DUBEY; SCHARES;
7 ORTEGA-MORA, 2007).
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25



26
27 **Fig 2.** Ciclo Biológico do *Neospora caninum.* Fonte: Adaptado de Dubey; Schares;
28 Ortega-Mora, 2007.
29
30
31 Os hospedeiros definitivos e intermediários podem se infectar por
32 duas principais vias, horizontal e vertical. A primeira ocorre quando oocistos
33 esporulados são ingeridos com água e alimentos contaminados ou por ingestão de
34 tecidos de hospedeiros intermediários contendo cistos teciduais. A segunda, também

chamada de transmissão transplacentária, é bastante comum nos bovinos e ocorre quando taquizoítos atravessam a barreira placentária e infectam o feto durante a gestação (DUBEY; SCHARES, 2006; DUBEY; SCHARES; ORTEGA-MORA, 2007). Vacas prenhes cronicamente infectadas podem ter uma re-agudização da doença, levando à uma disseminação de taquizoítas e consequente infecção fetal (WILLIAMS et al., 2009). Transmissão via lactogênea tem sido relatada experimentalmente bem como DNA do parasita tem sido descrito no sêmen de touros, entretanto essas duas vias são improváveis de acontecer em condições naturais (DAVISON et al., 2001; FERRE et al., 2005; DUBEY; SCHARES; ORTEGA-MORA, 2007).

O diagnóstico da neosporose é realizado por meio de exames sorológicos, já discutidos previamente, ou por meio de técnicas diretas que permitam a detecção do parasita ou DNA do mesmo, tal como o isolamento, métodos moleculares e imunohistoquímicos (DUBEY; SCHARES, 2006). O isolamento do parasita pode ser realizado em animais ou cultivo celular e é de difícil realização devido à baixa concentração de parasitas em animais naturalmente infectados, além dos custos elevados e questões bioéticas envolvidas (DUBEY; SCHARES; ORTEGA-MORA, 2007). O exame histopatológico deve ser utilizado como método auxiliar no diagnóstico pois apresenta baixa sensibilidade de detecção do parasita em cortes de tecidos, devendo ser sempre aliado com a imunohistoquímica, devido à utilização de anticorpos poli ou monoclonais para a identificação específica de antígenos parasitário (DUBEY; SCHARES, 2006).

Métodos moleculares como a PCR convencional e nested-PCR são técnicas de elevada sensibilidade e especificidade, sendo aplicáveis à uma ampla variedade de amostras biológicas. O gene Nc5 e a região do espaçador transcrito interno 1 (ITS-1) do RNA são os marcadores mais comumente utilizados para detecção do parasita, sendo o ITS-1 também utilizado para análises filogenéticas, pois apresenta regiões conservadas intraespécie e variáveis interespécies, permitindo assim a diferenciação entre outros coccídios (GONDIM et al., 2004a; DUBEY; SCHARES, 2006; DONAHOE et al., 2015). Outras técnicas moleculares como o mini e microsatélites estão disponíveis para identificação da variabilidade genética, o que pode determinar em uma variação na resposta imunológica ao parasita com consequente diferenças na patogenicidade da infecção (REGIDOR-CERRILLO et al., 2013).

Medidas de controle devem ser tomadas para evitar os prejuízos econômicos causados pela neosporose, principalmente em bovinos. Dentre essas medidas, são descritas a realização de exames sorológicos antes da introdução de animal no rebanho, evitar o acesso de cães à áreas de armazanamento de comida dos animais, descarte adequado de fetos abortados e restos placentários, prevenção da contaminação da água com fezes de canídeos, controle de roedores, transferência de embrião e abate de animais infectados (DUBEY; SCHARES; ORTEGA-MORA, 2007).

1 **REFERÊNCIAS**

2

3 ABREU, R.A.; WEISS, R.R.; THOMAZ-SOCCOL, V.; LOCATELLI-DITTRICH, R.;
4 LASKOSKI, L. M.; BERTOL, M.A.F.; KOCH, M.O.; ALBAN, S.M.; GREEN, K.T.
5 Association of antibodies against *Neospora caninum* in mares with reproductive
6 problems and presence of seropositive dogs as a risk factor. **Veterinary**
7 **Parasitology**, v. 202, n. 3-4, p. 128–131, 2014.

8 BAKER, D. G.; MORISHITA, T. Y.; BROOKS, D. L.; SHEN, S. K.; LINDSAY, D. S.;
9 DUBEY, J. P. Experimental oral inoculations in birds to evaluate potential definitive
10 hosts of *Neospora caninum*. **The Journal of parasitology**, v. 81, n. 5, p. 783–785,
11 1995.

12 BARR, B. C.; ANDERSON, M. L.; DUBEY, J. P.; CONRAD, P. A. *Neospora*-like
13 protozoal infections associated with bovine abortions. **Veterinary pathology**, v. 28,
14 n. 2, p. 110–116, 1991.

15 BARROS, L. D.; TARODA, A.; ZULPO, D. L.; CUNHA, I. A. L.; SAMMI, A. S.;
16 CARDIM, S. T.; MIURA, A. C.; SU, C.; MACHADO, R. Z.; VIDOTTO, O.; GARCIA, J.
17 L. Genetic characterization of *Toxoplasma gondii* isolates from eared doves (Z*enaida*
18 *auriculata*) in Brazil. **Revista brasileira de parasitologia veterinária** v. 23, n. 4, p.
19 443–448, 2014.

20 BIEŃ, J.; MOSKWA, B.; CABAJ, W. In vitro isolation and identification of the first
21 *Neospora caninum* isolate from European bison (*Bison bonasus* bonasus L.).
22 **Veterinary Parasitology**, v. 173, n. 3-4, p. 200–205, 2010.

23 BJERKÅS, I.; MOHN, S. F.; PRESTHUS, J. Unidentified cyst-forming sporozoon
24 causing encephalomyelitis and myositis in dogs. **Zeitschrift fur Parasitenkunde**
25 **(Berlin, Germany)**, v. 70, n. 2, p. 271–274, 1984.

26 BJERKAS, I.; PRESTHUS, J. Immuno-histochemical and ultrastructural
27 characteristics of a cyst-forming sporozoon associated with encephalomyelitis and
28 myositis in dogs. **Apmis**, v. 96, n. 5, p. 445–454, 1988.

29 BJÖRKMAN, C.; UGGLA, A. Serological diagnosis of *Neospora caninum* infection.
30 **International Journal for Parasitology**, v. 29, n. 10, p. 1497–1507, 1999.

31 BUCHER, E. H.; RANVAUD, R. D. S31-4 Eared dove outbreaks in South America :
32 patterns and characteristics. **Acta Zoologica Sinica**, v. 52, p. 564–567, 2006.

33 CAMPERO, L. M.; VENTURINI, M. C.; MOORE, D. P.; MASSOLA, L.;
34 LAGOMARSINO, H.; GARCIA, B.; BACIGALUPE, D.; RAMBEAUD, M.; PARDINI, L.;
35 LEUNDA, M. R.; SCHARES, G.; CAMPERO, C. M. Isolation and molecular
36 characterization of a new *Neospora caninum* isolate from cattle in Argentina.
37 **Experimental parasitology**, v. 155, p. 8–12, 2015.

38 COSTA, K. S.; SANTOS, S. L.; UZÊDA, R. S.; PINHEIRO, A. M.; ALMEIDA, M. A.
39 O.; ARAÚJO, F. R.; MCALLISTER, M. M.; GONDIM, L. F. P. Chickens (G*allus*
40 *domesticus*) are natural intermediate hosts of *Neospora caninum*. **International**
41 **journal for parasitology**, v. 38, n. 2, p. 157–159, 2008.

DAVISON, H. C.; GUY, C. S.; MCGARRY, J. W.; GUY, F.; WILLIAMS, D. J. L.; KELLY, D. F.; TREES, A. J. Experimental studies on the transmission of *Neospora caninum* between cattle. **Research in Veterinary Science**, v. 70, n. 2, p. 163–168, 2001.

DIAZ, L. A.; OCCELLI, M.; ALMEIDA, F. L.; ALMIRÓN, W. R.; CONTIGIANI, M. S. Eared dove (*Zenaida auriculata*, Columbidae) as host for St. Louis encephalitis virus (Flaviviridae, Flavivirus). **Vector borne and zoonotic diseases**, v. 8, n. 2, p. 277–282, 2008.

DONAHOE, S. L.; LINDSAY, S. a.; KROCKENBERGER, M.; PHALEN, D.; ŠLAPETA, J. A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. **International Journal for Parasitology: Parasites and Wildlife**, v. 4, n. 2, p. 216–238, 2015.

DUBEY, J.P.; BARR, B.C.; BARTA, J.R.; BJERKÅS, I.; BJÖRKMAN, C.; BLAGBURN, B.L.; BOWMAN, D.D.; BUXTON, D.; ELLIS, J.T.; GOTTSTEIN, B.; HEMPHILL, A.; HILL, D.E.; HOWE, D.K.; JENKINS, M.C.; KOBAYASHI, Y.; KOUDELA, B.; MARSH, A E.; MATTSSON, J.G.; MCALLISTER, M.M.; MODRÝ, D.; OMATA, Y.; SIBLEY, L.D.; SPEER, C.A..; TREES, A.J.; UGGLA, A.; UPTON, S.J.; WILLIAMS, D.J.L.; LINDSAY, D.S. Redescription of *Neospora caninum* and its differentiation from related coccidia. **International Journal for Parasitology**, v. 32, n. 8, p. 929–946, 2002.

DUBEY, J. P.; HATTEL, A. L.; LINDSAY, D. S.; TOPPER, M. J. Neonatal N*eospora caninum* infection in dogs: isolation of the causative agent and experimental transmission. **Journal of the American Veterinary Medical Association**, v. 193, n. 10, p. 1259–63, 1988.

DUBEY, J. P.; JENKINS, M. C.; FERREIRA, L. R.; CHOUDHARY, S.; VERMA, S. K.; KWOK, O. C. H.; FETTERER, R.; BUTLER, E.; CARSTENSEN, M. Isolation of viable *Neospora caninum* from brains of wild gray wolves (*Canis lupus*). **Veterinary parasitology**, v. 201, n. 1-2, p. 150–153, 2014.

DUBEY, J. P.; JENKINS, M. C.; RAJENDRAN, C.; MISKA, K.; FERREIRA, L. R.; MARTINS, J.; KWOK, O. C. H.; CHOUDHARY, S. Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. **Veterinary Parasitology**, v. 181, n. 2-4, p. 382–387, 2011.

DUBEY, J. P.; RIGOULET, J.; LAGOURETTE, P.; GEORGE, C.; LONGEART, L.; LENET, J. L. Fatal transplacental neosporosis in a deer (*Cervus eldi siamensis*). **The Journal of parasitology**, v. 82, n. 2, p. 338–339, 1996.

DUBEY, J. P.; SCHARES, G. Diagnosis of bovine neosporosis. **Veterinary Parasitology**, v. 140, n. 1-2, p. 1–34, 2006.

DUBEY, J. P.; SCHARES, G. Neosporosis in animals-The last five years. **Veterinary Parasitology**, v. 180, n. 1-2, p. 90–108, 2011.

DUBEY, J. P.; SCHARES, G.; ORTEGA-MORA, L. M. Epidemiology and Control of Neosporosis and *Neospora caninum*. **Clinical Microbiology Reviews**, v. 20, n. 2, p. 323–367, 2007.

1 DUBEY, J. P.; SREEKUMAR, C.; KNICKMAN, E.; MISKA, K. B.; VIANNA, M. C. B.;
2 KWOK, O. C. H.; HILL, D. E.; JENKINS, M. C.; LINDSAY, D. S.; GREENE, C. E.
3 Biologic, morphologic, and molecular characterisation of *Neospora caninum* isolates
4 from littermate dogs. **International Journal for Parasitology**, v. 34, n. 10, p. 1157–
5 1167, 2004.

6 FEITOSA, T. F.; VILELA, V. L. R.; BEZERRA DE MELO, L. R.; DE ALMEIDA NETO,
7 J. L.; DE OLIVEIRA SOUTO, D. V.; DE MORAIS, D. F.; ATHAYDE, A. C. R.;
8 AZEVEDO, S. S.; DE JESUS PENA, H. F. *Toxoplasma gondii* and *Neospora*
9 *caninum* in slaughtered pigs from Northeast, Brazil. **Veterinary Parasitology**, v. 202,
10 n. 3-4, p. 305–309, 2014.

11 FERRE, I.; ADURIZ, G.; DEL-POZO, I.; REGIDOR-CERRILLO, J.; ATXAERANDIO,
12 R.; COLLANTES-FERNÁNDEZ, E.; HURTADO, A.; UGARTE-GARAGALZA, C.;
13 ORTEGA-MORA, L. M. Detection of *Neospora caninum* in the semen and blood of
14 naturally infected bulls. **Theriogenology**, v. 63, n. 5, p. 1504–1518, 2005.

15 GONDIM, L. F. P.; LASKI, P.; GAO, L.; MCALLISTER, M. M. Variation of the internal
16 transcribed spacer 1 sequence within individual strains and among different strains of
17 *Neospora caninum*. **The Journal of parasitology**, v. 90, n. 1, p. 119–122, 2004a.

18 GONDIM, L. F. P.; MCALLISTER, M. M.; PITT, W. C.; ZEMLICKA, D. E. Coyotes
19 (*Canis latrans*) are definitive hosts of *Neospora caninum*. v. 34, p. 159–161, 2004b.

20 GONDIM, L. S. Q.; ABE-SANDES, K.; UZÊDA, R. S.; SILVA, M. S. A.; SANTOS, S.
21 L.; MOTA, R. A.; VILELA, S. M. O.; GONDIM, L. F. P. *Toxoplasma gondii* and
22 *Neospora caninum* in sparrows (*Passer domesticus*) in the Northeast of Brazil.
23 **Veterinary parasitology**, v. 168, n. 1-2, p. 121–124, 2010.

24 KING, J. S.; ŠLAPETA, J.; JENKINS, D. J.; AL-QASSAB, S. E.; ELLIS, J. T.;
25 WINDSOR, P. A. Australian dingoes are definitive hosts of *Neospora caninum.*
26 **International Journal for Parasitology**, v. 40, n. 8, p. 945–950, 2010.

27 KOYAMA, T.; KOBAYASHI, Y.; OMATA, Y.; YAMADA, M.; FURUOKA, H.; MAEDA,
28 R.; MATSUI, T.; SAITO, A.; MIKAMI, T. Isolation of *Neospora caninum* from the
29 brain of a pregnant sheep. **The Journal of parasitology**, v. 87, n. 6, p. 1486–1488,
30 2001.

31 LINDSAY, D. S.; UPTON, S. J.; DUBEY, J. P. A structural study of the *Neospora*
32 *caninum* oocyst. **International Journal for Parasitology**, v. 29, n. 10, p. 1521–1523,
33 1999.

34 LOBATO, J.; SILVA, D. A. O.; MINEO, T. W. P.; AMARAL, J. D. H. F.; SILVA
35 SEGUNDO, G. R.; COSTA-CRUZ, J. M.; FERREIRA, M. S.; BORGES, A. S.;
36 MINEO, J. R. Detection of immunoglobulin G antibodies to *Neospora caninum* in
37 humans: High seropositivity rates in patients who are infected by human
38 immunodeficiency virus or have neurological disorders. **Clinical and Vaccine**
39 **Immunology**, v. 13, n. 1, p. 84–89, 2006.

40 MARTINS, J.; KWOK, O. C. H.; DUBEY, J. P. Seroprevalence of *Neospora caninum*
41 in free-range chickens (*Gallus domesticus*) from the Americas. **Veterinary**
42 **Parasitology**, v. 182, n. 2-4, p. 349–351, 2011.

MCALLISTER, M. M.; DUBEY, J. P.; LINDSAY, D. S.; JOLLEY, W. R.; WILLS, R. A.; MCGUIRE, A. M. Dogs are definitive hosts of *Neospora caninum*. **International Journal for Parasitology**, v. 28, n. 9, p. 1473–1478, 1998.

MENESES, I. D.; ANDRADE, M. R.; UZÊDA, R. S.; BITTENCOURT, M. V; LINDSAY, D. S.; GONDIM, L. F. Frequency of antibodies against *Sarcocystis neurona* and *Neospora caninum* in domestic cats in the state of Bahia, Brazil. **Rev Bras Parasitol Vet**, v. 23, p. 526–529, 2014.

MORÉ, G.; PARDINI, L.; BASSO, W.; MARÍN, R.; BACIGALUPE, D.; AUAD, G.; VENTURINI, L.; VENTURINI, M. C. Seroprevalence of *Neospora caninum*, *Toxoplasma gondii* and *Sarcocystis* sp. in llamas (*Lama glama*) from Jujuy, Argentina. **Veterinary Parasitology**, v. 155, n. 1-2, p. 158–160, 2008.

NASCIMENTO, C. O. M.; SILVA, M. L. C. R.; KIM, P. C. P.; GOMES, A. A. B.; GOMES, A. L. V; MAIA, R. C. C.; ALMEIDA, J. C.; MOTA, R. A. Occurrence of *Neospora caninum* and *Toxoplasma gondii* DNA in brain tissue from hoary foxes (*Pseudalopex vetulus*) in Brazil. **Acta Tropica**, v. 146, p. 60–65, 2015.

OSHIRO, L. M.; MOTTA-CASTRO, A. R. C.; FREITAS, S. Z.; CUNHA, R. C.; DITTRICH, R. L.; MEIRELLES, A. C. F.; ANDREOTTI, R. *Neospora caninum* and *Toxoplasma gondii* serodiagnosis in human immunodeficiency virus carriers. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 48, n. 5, p. 568–572, 2015.

RANVAUD, R.; DE FREITAS, K. C.; BUCHER, E. H.; DIAS, H. S.; AVANZO, V. C.; ALBERTS, C. C. Diet of eared doves (*Zenaida auriculata*, Aves, Columbidae) in a sugar-cane colony in South-eastern Brazil. **Brazilian journal of biology**, v. 61, n. 4, p. 651–660, 2001.

REGIDOR-CERRILLO, J.; DÍEZ-FUERTES, F.; GARCÍA-CULEBRAS, A.; MOORE, D. P.; GONZÁLEZ-WARLETA, M.; CUEVAS, C.; SCHARES, G.; KATZER, F.; PEDRAZA-DÍAZ, S.; MEZO, M.; ORTEGA-MORA, L. M. Genetic diversity and geographic population structure of bovine *Neospora caninum* determined by microsatellite genotyping analysis. **PLoS ONE**, v. 8, n. 8, p. 1–12, 2013.

REICHEL, M. P.; ALEJANDRA AYANEGUI-ALCÉRRECA, M.; GONDIM, L. F. P.; ELLIS, J. T. What is the global economic impact of *Neospora caninum* in cattle - the billion dollar question. **International journal for parasitology**, v. 43, n. 2, p. 133–42, 2013.

REICHEL, M. P.; MCALLISTER, M. M.; NASIR, A.; MOORE, D. P. A review of *Neospora caninum* in water buffalo (*Bubalus bubalis*). **Vet Parasitol**, v. 212, n. 3-4, p. 75–79, 2015.

ROBBE, D.; PASSARELLI, A.; GLORIA, A.; DI CESARE, A.; CAPELLI, G.; IORIO, R.; TRAVERSA, D. *Neospora caninum* seropositivity and reproductive risk factors in dogs. **Experimental parasitology**, v. 164, p. 31–35, 2016.

RODRIGUES, A. A. R.; GENNARI, S. M.; AGUIAR, D. M.; SREEKUMAR, C.; HILL, D. E.; MISKA, K. B.; VIANNA, M. C. B.; DUBEY, J. P. Shedding of *Neospora caninum* oocysts by dogs fed tissues from naturally infected water buffaloes (*Bubalus bubalis*) from Brazil. v. 124, p. 139–150, 2004.

ROMANELLI, P. R.; FREIRE, R. L.; VIDOTTO, O.; MARANA, E. R. M.; OGAWA, L.; DE PAULA, V. S. O.; GARCIA, J. L.; NAVARRO, I. T. Prevalence of *Neospora caninum* and Toxoplasma gondii in sheep and dogs from Guarapuava farms, Paraná State, Brazil. **Research in Veterinary Science**, v. 82, n. 2, p. 202–207, 2007.

SADREBAZZAZ, A.; HADDADZADEH, H.; SHAYAN, P. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in camels (*Camelus dromedarius*) in Mashhad, Iran. **Parasitology research**, v. 98, n. 6, p. 600–1, 2006.

SALANT, H.; MAZUZ, M. L.; SAVITSKY, I.; NASEREDDIN, A.; BLINDER, E.; BANETH, G. *Neospora caninum* in crows from Israel. **Veterinary Parasitology**, v. 212, n. 3-4, p. 375–378, 2015.

SHIBATTA, O. A.; GALVES, W.; CARMO, W. P. D. do; LIMA, I. P.; LOPES, E. V.; MACHADO, R. A. A fauna de vertebrados do campus da Universidade Estadual de Londrina, região norte do estado do Paraná, Brasil. **Semina: Ciências Biológicas e da Saúde**, v. 30, n. 1, p. 3–26, 2009.

SICK, H. **Ornitologia brasileira**. 3. ed. Rio de Janeiro: Editora Nova Fronteira, 2001.

SOUSA, M. E.; PORTO, W. J. N.; ALBUQUERQUE, P. P. F.; SOUZA NETO, O. L.; FARIA, E. B.; PINHEIRO JÚNIOR, J. W.; MOTA, R. A. Seroprevalence and risk factors associated with infection by *Neospora caninum* of dairy cattle in the state of Alagoas, Brazil. **Pesquisa Veterinaria Brasileira**, v. 32, n. 10, p. 1009–1013, 2012.

SOUZA, E. A; TELINO-JÚNIOR, W. R.; NASCIMENTO, J. L. X.; LYRA-NEVES, R. M.; AZEVEDO JÚNIOR, S. M.; FILHO, C. L.; NETO, A. S. Estimativas populacionais de avoantes *Zenaida auriculata* (Aves Columbidae, DesMurs, 1847) em colônias reprodutivas no Nordeste do Brasil. **Ornithologia**, v. 2, n. 1, p. 28–33, 2007.

TOPAZIO, J. P.; WEBER, A.; CAMILLO, G.; VOGEL, F. F.; MACHADO, G.; RIBEIRO, A.; MOURA, A.B.; LOPES, L. S.; TONIN, A.A.; SOLDÁ, N. M.; BRÄUNIG, P.; SILVA, A.S. Seroprevalence and risk factors for *Neospora caninum* in goats in Santa Catarina state, Brazil. **Revista Brasileira Parasitologia Veterinaria**, v. 23, p. 360–366, 2014.

TRANAS, J.; HEINZEN, R. A.; WEISS, L. M. Serological Evidence of Human Infection with the Protozoan *Neospora caninum*. **Clinical and Diagnostic Laboratory Immunology** v. 6, n. 5, p. 765–767, 1999.

VIANNA, M. C. B.; SREEKUMAR, C.; MISKA, K. B.; HILL, D. E.; DUBEY, J. P. Isolation of *Neospora caninum* from naturally infected white-tailed deer (*Odocoileus virginianus*). **Veterinary Parasitology.** v. 129, p. 253–257, 2005.

WILLIAMS, D. J. L.; HARTLEY, C. S.; BJÖRKMAN, C.; TREES, A.J. Endogenous and exogenous transplacental transmission of *Neospora caninum* - how the route of transmission impacts on epidemiology and control of disease. **Parasitology**, v. 136, n. 14, p. 1895–1900, 2009.

3 ARTIGO DE REVISÃO PARA PUBLICAÇÃO**

NEOSPORA CANINUM IN BIRDS: A REVIEW

** Artigo redigido sob as normas de publicação do periódico Veterinary Parasitology, disponível em: https://www.elsevier.com/journals/veterinary-parasitology/0304-4017?generatepdf=true Acesso: 13/02/2016

Abstract

Neospora caninum is an obligate intracellular protozoan parasite that infects domestic and wild animals. Canids are considered definitive hosts since they may shed oocysts in the environment by feces. The disease is recognized as one of the major causes of bovine abortion worldwide, leading to important economic losses in dairy and beef cattle. Previously studies have been reported *N. caninum* infection in different species of birds, what have been associated with increase in seroprevalence and reproductive problems in dairy cattle. Although the role of birds in the epidemiological cycle of neosporosis in unknown, these animals are exposed to infection, since they feed on the ground, and could contribute to parasite dissemination. This review is focused on current status of neosporosis in birds.

Keywords: *Neospora caninum; neosporosis; birds*

1. Introduction
2. Serological studies
3. Experimental infections
4. Natural infections
5. Conclusions

1. Introduction

Neospora caninum is a protozoan parasite that was first described by Bjerkås et al. (1984), who had reported dogs with cysts-forming sporozoon associated with lesions in the central nervous system and skeletal muscles. Since it shares

morphological similarity with *T. gondii*, *N. caninum* has been misdiagnosed until 1988 however Bjerkås and Presthus (1988) showed by immuno-histochemical and ultrastructural characteristics of cysts, they were different. Due to these discoveries, *N.* caninum was formally recognized as a new genus and species (Dubey et al., 1988a).

The life cycle of *N. caninum* was unclear at the beginning and an experimental study with carnivorous birds was performed to determine if birds were definitive host of *N. caninum* (Baker et al., 1995). This study used the red-tailed hawks (*Buteo* jamaicensis), turkey vultures (*Cathartes aura*), barn owls (*Tyto alba*) and the american crows (*Corvus brachyrhynchus*), that were fed with mice that had been inoculated subcutaneously with 10^5 culture-derived tachyzoites of *N. caninum*. Although occasional unsporulated oocysts were observed in fecal samples, none of these were similar to *N. caninum* and mice inoculated with these oocysts did not show either antibodies against *N. caninum* or tissue cysts in the brain.

A few years later, McAllister et al. (1998) showed that dogs (*Canis familiaris*) are definitive hosts of the parasite, since a sexual phase occurs in the intestine of these animals shedding oocysts in their feces. After the description of dogs as definitive hosts of the *N. caninum* life cycle, other studies showed that canids, including the Australian dingo (*Canis lupus dingo*) (King et al., 2010), the coyote (*Canis latrans*) (Gondim et al., 2004) and the gray wolf (*Canis lupus*) (Dubey et al., 2011) are definitive hosts and can shed oocysts in their feces. Afterwards, Dubey et al. (2002) based mainly on morphologic aspects, made a redescription of the parasite and a differentiation of related parasites.

In the past years, many studies using molecular techniques have been used to show that small mammals and birds are intermediate hosts of *N. caninum*

(Čobádiová et al., 2013; Costa et al., 2008; Du et al., 2015; Ferroglio et al., 2007; Fuehrer et al., 2010; Gondim et al., 2010; Hughes et al., 2008; Truppel et al., 2010). However, viable parasite has been isolated only in dog (Dubey et al., 1998), gray wolf (Dubey et al., 2014), cattle (Campero et al., 2015), sheep (Pena et al., 2007), water buffalo (Rodrigues et al., 2004), bison (Bień et al., 2010) and white-tailed deer (Vianna et al., 2005). Antibodies against this parasite has been described in many hosts, even in human, however the zoonotic potential is uncertain since the parasite has not been detected in human tissues (Tranas et al., 1999)

Although the role of birds in the life cycle of *N. caninum* is not completely understood, previously studies have shown that the presence of birds on dairy farms could increase the seroprevalence and reproductive problems associated to *N. caninum*, suggesting that these animals may be an important intermediate host, contributing to transmission of the parasite to definitive hosts (Bartels et al., 1999; Otranto et al., 2003). Thus, the present manuscript provides an overview on what is known about *N. caninum* in birds.

2. Serological studies

There are few serological studies to detect anti-*N. caninum* antibodies in birds. The first one was performed by Costa et al. (2008), which using indirect fluorescent antibody tests (IFAT) observed a seroprevalence of 12.5 % (50/400) in chickens from Bahia state, Brazil. However, when compared the origin of these animals, the prevalence was higher in outdoors chickens (23.5%, 47/200) than indoor (1.5%, 3/200), showing that chickens raised outside are more exposed to *N. caninum* and may contribute to the parasite transmission. Gonçalves et al. (2012) also working

1 with chickens from Brazil have observed a prevalence of 17% (17/100) using IFAT
2 and a cut off of 50, moreover, an significant agreement between *T. gondii* and *N.*
3 *caninum* serology was observed, indicating that birds can may have contact with
4 both coccidian parasites.

5 Afterwards, a serological study with wild and captive birds was performed
6 (Mineo et al., 2011). The authors used 294 birds, belonging to nine different orders,
7 but no serological positivity was observed in all tested samples. Although they did not
8 observed antibodies against *N. caninum* in sera from these animals, Apicomplexa-
9 like cysts immunostained with *N. caninum* antisera were found in cloacae and
10 cervical musculature from two Psittaciformes.

11 In the same year, Martins et al. (2011) performed a serological study of *N.*
12 *caninum* in free-range chickens (*Gallus domesticus*) from the Americas. Birds from
13 previous studies with *T. gondii* from different countries of North, Central and South
14 America were used. According with IFAT and a cut off of 25, antibodies against the
15 parasite was found in 39.5% (524/1324) of birds, showing that free-range chickens
16 have been exposed to *N. caninum.*

17 Molina-López et al. (2012) observed a high prevalence of antibodies against
18 *N. caninum* in common rave (*Corvus corax*) from Northeast of Spain. Using IFAT and
19 a cut off of 50, 35.8% (24/67) of animals were positive, with titers ranging from 50
20 until 100. A recent study with free ranging crows was done in Israel (Salant et al.,
21 2015). A total of 183 birds belonging to the species *Corvus cornix*, *Corvus monedula*
22 and *Corvus splendes* were used to evaluate the exposure of these animals to *N.*
23 *caninum* by means of modified agglutination test (MAT) and IFAT. Thirty out 183
24 animals (16.4%) were positive in both tests, showing that crows (*C. cornix* and *C.*

1 *monedula*) are exposed to *N. caninum* and could be an important role in the
2 epidemiology of the parasite.
3 Recently, Camillo et al. (2015) performed a study with backyard chickens,
4 since they may be good bioindicator of the environmental contamination with oocysts.
5 One hundred and thirty-seven animals from 23 different farms from Rio Grande do
6 Sul state, Brazil were tested using IFAT and cut off of 50. Antibodies anti-*N. caninum*
7 were detected in 36.5% (50/137), indicating a widespread exposure of backyard
8 chickens to the parasite in that region.
9 A summary of seroprevalence studies of *N. caninum* in birds is shown in Table
10 1.
11
12 **3. Experimental infections**
13
14 The first experimental study with *N. caninum* and birds was performed by
15 Baker et al. (1995) when definitive host of the parasite was unknown. After the
16 discovery that canids are definitive hosts, some experimental studies were done to
17 evaluate the potential of birds as intermediate hosts in the life cycle. In the past, only
18 cattle were known as intermediate host of *N. caninum*, but since serological studies
19 have provided evidence of infection in wild canids, McGuirre et al. (1999) proposed
20 that *N. caninum* would have an intermediate host that is common pray of those wild
21 canids. Thus, the authors designed an experimental study with inoculation of *N.*
22 *caninum* tachyzoites in pigeons (*Columba livia*) and zebra finches (*Poephila guttata*).
23 Three animals from each species were inoculated intraperitoneally with different
24 doses (10^6, 10^5 and 10^4) of two different strains (NC-2 and NC-Liverpool) of the
25 parasite. By means of IFAT, tissue culture and PCR, all pigeons were positive and

1 none of zebra finches were. Although only one pigeon was positive in the histological
2 examination, typical lesions of neosporosis were observed. These results have
3 suggested that Columbiform birds could be a good candidate for intermediate host
4 and could play an important role in the biological cycle.

5 Furuta et al. (2007) realized an experimental infection in chickens and
6 embryonated eggs with *N. caninum*. Laying hens, 7-day-old chicks and embryonated
7 eggs were inoculated with different parasite concentration. None of chicks showed
8 clinical signs of infection but they had seroconversion, with titer of 400 on IFAT 15
9 days post-infection (d.p.i.). Immunohistochemistry analysis also showed the parasite
10 in different organs, showing disseminated acute infection. Laying hens showed a
11 similar pattern to chickens in serological response, with positive result on IFAT 15
12 d.p.i. and non-detectable antibodies at 60 d.p.i. None evidence of vertical
13 transmission was observed, but embryonated eggs infected by through allantoic
14 cavity had susceptibility to infection with high mortality rate. Dogs that had received
15 inoculated eggs produced serological response and shed oocysts by feces, showing
16 that chickens could be an intermediate host of *N. caninum*.

17 In the same line of research, an experimental study with broiler chicken
18 embryonated eggs was conducted (Mansourian et al., 2009). Embryonated eggs
19 were inoculated with different tachyzoites concentrations (from 10 to 10^6) via
20 chorioallantoic. None hatching was observed in the groups with the highest parasite
21 concentration, while in the groups inoculated with low parasite concentration,
22 hatching occurred, but one chicken showed neurological signs such as hind limb
23 paralysis, pedaling movements, lack of coordination, while other three birds had
24 arthritis in the feet joints.

1 The potential of quails (*Coturnix coturnix japonica*) as an intermediate host of
2 *N. caninum* was also evaluated (de Oliveira et al., 2013). Animals were divided in
3 three groups and received different concentrations of parasite ($3.5x10^6$, $5x.10^6$ and
4 placebo group) subcutaneously. None of the quails showed clinical signs of infection
5 or died during the experiment. By means of IFAT and a cut off of 10, seroconversion
6 occurred on 7 d.p.i., with peak on 14 d.p.i. Mononuclear inflammatory infiltrate,
7 immunoreactivity by IHC and molecular detection was observed mainly during the
8 first two weeks of infection. None oocyst was found in feces from dogs that were fed
9 with inoculated quails, no molecular detection of dog stool samples and no dog
10 seroconversion was observed, demonstrating that quails are not a good intermediate
11 host for *N. caninum*.
12 Munhoz et al. (2014) analyzed the susceptibility of *Gallus gallus domesticus* to
13 *N. caninum* tachyzoites infection. For this, two experiments were done. In the first
14 one, poultries were divided in groups that received feed with and without coccidiostat,
15 followed by subcutaneous inoculation of $3x10^6$ tachyzoites. No evidence of infection
16 in adults and chicks were observed by IHC and histopathology. It also was not
17 observed DNA amplification in infected bird tissues and no dogs that were fed by
18 tissues from birds shed oocyst by feces. In another experiment, embryonated chicken
19 eggs were challenged with $1x10^2$ tachyzoites. Chicks born from these eggs were
20 euthanized and again, no lesion compatible with neosporosis, no DNA amplification
21 and no oocysts shed by dogs were observed, indicating that the parasite could have
22 been eliminated by the host.
23
24 **4. Natural infections**
25

1 Although antibodies against *N. caninum* have been described in many
2 animals, such as canids, felids, cervids, ruminants, birds and marine mammals, just
3 few of these are considered as intermediate host. The first study that showed that
4 birds are intermediate host for *N. caninum* was done by Costa et al. (2008). Brain
5 tissue from 10 positive chickens by serological test was used to DNA extraction
6 followed by PCR. Cloning of PCR fragments and sequencing were also performed.
7 Six animals (60%) from 10 seropositive were positive by PCR for *N. caninum* and
8 negative for *T. gondii*, showing for the first time a natural *N. caninum* infection in
9 birds. In another study with natural infection of chickens from Brazil, DNA from *N.*
10 *caninum* was detected in 6% of animals, using brain and heart tissue (Gonçalves et
11 al., 2012). By means of nested-PCR based on ITS-1 region followed by sequencing,
12 the sequences showed 99-100% of identity with previously data. Furthermore, all
13 animals positive by PCR were negative at serological tests, showing that birds can be
14 infected but seronegative, probably due to decrease of specific circulating antibodies
15 in infected birds as previously demonstrated by Mineo et al. (2009).
16 Afterwards, Gondim et al. (2010) showed *N. caninum* infection in sparrows
17 (*Passer domesticus*) from Brazil. Heart and brain of 40 animals from Pernambuco
18 and Bahia states were used for DNA extraction followed by a nested PCR using
19 primers to conserved region of internal transcribe spacer (ITS1) between *Hammondia*
20 sp., *N. caninum* and *T. gondii.* Sequentially, sequencing was performed to identify the
21 parasite. In the nested-PCR, 25% (10/40) of samples were positive for
22 Toxoplasmatinae, however, 30% of those positive samples were classified as *N.*
23 *caninum* by DNA sequencing. It was the first study that showed that wild birds may
24 act as intermediate host of *N. caninum,* indicating an importance of these birds in
25 epidemiological cycle, since sparrows, may be prey by canids, contributing to

transmission of the parasite. Abdoli et al. (2015) have also detected *N. caninum* in sparrows from Iran by molecular tests. Brain tissue from 217 birds were used for DNA extraction and nested-PCR using primers Np21 plus and Np6plus in the first reaction and Np7 and Np6 in the second. DNA from *N. caninum* was observed in eight birds (3.68%) and sequence analyses showed 97-99% of similarity with other isolates deposited in GenBank.

The presence of tissue cysts in wild birds was previously described (Mineo et al., 2011). Analysis of two birds, one red-and-green macaw (*Ara chloropterus*) and other blue-fronted Amazon parrots (*Amazona aestiva*), that had died during internment in a veterinary hospital, showed Apicomplexan-like tissue cysts in HE stained from the cloacae of the first bird and cervical of the second. Immunohistochemistry analysis of these samples revealed positive immunostaining with anti-*N. caninum* polyclonal antibodies. This first report of latent parasitic forms in the musculature of wild birds brought some important points to discussion of *Neospora* life cycle in wildlife animals.

Neospora caninum DNA has also been detected in brain tissue from wild birds of Spain (Darwich et al., 2012). Brain tissue from 201 animals belonging to 14 species from three different regions of Spain was used for DNA extraction and PCR test using the primers Np21-plus and Np6-plus, targeting genomic Nc5 region. Only three (1.5%) samples were positive for *N. caninum* DNA, two from magpies (*Pica pica*) and one from common buzzard (*Buteo buteo*), showing 98% of identify with previously *N. caninum* sequences described and deposited in GenBank. The detection of *N. caninum* in these birds should be considered important for the epidemiologic cycle, since common buzzard is a common predator and usually ingest carrion, which could contribute to infection of these animals.

1 A study with crows of three different species from different areas of Israel was
2 conducted to confirm if these animals were an intermediate host of *N. caninum*
3 (Salant et al., 2015). Nested-PCR targeting Nc5 gene was carried out to detect the
4 DNA of the parasite in the brain tissue from crows. Only two animals (1.09%)
5 belonging to the species *Corvus cornix* and *Corvus monedula*, were found to be
6 positive by nPCR, showing 99% of identify with DNA sequences previously
7 described. Interesting, both positive samples were from animals seronegative by
8 means of MAT and IFAT assay, which point for the importance of the molecular test
9 since some birds may be present an undetectable level of antibodies after infection
10 with *N. caninum*.

11 Recently, DNA of the parasite was described in pigeons from China (Du et al.,
12 2015) Brain tissue of 210 pigeons from pigeons farms were collected for DNA
13 extraction. DNA detection of parasite was performed by nested-PCR using primers
14 for specific Nc5 gene while ITS-1 region were used to sequencing analysis. PCR
15 positive result was observed in 63 (30%) and ITS-1 analysis showed the presence of
16 clusters with 42 samples assigned to the NC-PR genotype, 10 to NC-1 genotype and
17 5, 3 and 3 to new genotypes identified. This was the first report of *N. caninum* DNA in
18 tissues from pigeons, which expands the list of the intermediate host of *N. caninum*
19 and point for the importance of these animals in the transmission of the parasite.

20 A summary of all birds considered intermediate hosts of *N. caninum* is shown
21 in Table 2 while other species in Table 3.
22
23
24
25

1 **5. Conclusions**

2

3 Nowadays, molecular studies have shown that *N. caninum* has a large range
4 of intermediate host, which contributes to elucidate the epidemiology of the disease.
5 Birds in special may have contribute to dissemination of the parasite, mainly because
6 these animals usually feed on the ground, becoming expose to many pathogens, and
7 are preyed upon by canids, contributing to the life cycle of the parasite.
8 Further studies with different species of birds are needed to elucidate the real
9 importance of birds in the epidemiology of neosporosis, aiming control the disease
10 and decrease the economic impact.

11

12 **References**

13

14 Abdoli, A., Arbabi, M., Dalimi, A., Pirestani, M., 2015. Molecular detection of
15 *Neospora caninum* in house sparrows (*Passer domesticus*) in Iran. Avian Pathol.
16 44, 319–322.
17 Baker, D.G., Morishita, T.Y., Brooks, D.L., Shen, S.K., Lindsay, D.S., Dubey, J.P.,
18 1995. Experimental oral inoculations in birds to evaluate potential definitive hosts
19 of *Neospora caninum*. J. Parasitol. 81, 783–785.
20 Bartels, C.J., Wouda, W., Schukken, Y.H., 1999. Risk factors for *Neospora caninum*-
21 associated abortion storms in dairy herds in The Netherlands (1995 to 1997).
22 Theriogenology 52, 247–57.
23 Bień, J., Moskwa, B., Cabaj, W., 2010. In vitro isolation and identification of the first
24 *Neospora caninum* isolate from European bison (*Bison bonasus bonasus* L.).
25 Vet. Parasitol. 173, 200–205.

1 Bjerkås, I., Mohn, S.F., Presthus, J., 1984. Unidentified cyst-forming sporozoon
2 causing encephalomyelitis and myositis in dogs. Z. Parasitenkd. 70, 271–274.
3 Bjerkas, I., Presthus, J., 1988. Immuno-histochemical and ultrastructural
4 characteristics of a cyst-forming sporozoon associated with encephalomyelitis
5 and myositis in dogs. Apmis 96, 445–454.
6 Camillo, G., Cadore, G., Ferreira, M., Braünig, P., Maciel, J., Pivoto, F., Sangioni, L.,
7 Vogel, F., 2015. *Toxoplasma gondii* and *Neospora caninum* antibodies in
8 backyard chickens in Rio Grande do Sul, Brazil. Rev. Bras. Ciência Avícola 17,
9 263–265.
10 Campero, L.M., Venturini, M.C., Moore, D.P., Massola, L., Lagomarsino, H., Garcia,
11 B., Bacigalupe, D., Rambeaud, M., Pardini, L., Leunda, M.R., Schares, G.,
12 Campero, C.M., 2015. Isolation and molecular characterization of a new
13 *Neospora caninum* isolate from cattle in Argentina. Exp. Parasitol. 155, 8–12.
14 Čobádiová, a, Víchová, B., Majláthová, V., Reiterová, K., 2013. First molecular
15 detection of *Neospora caninum* in European brown bear (*Ursus arctos*). Vet.
16 Parasitol. 197, 346–349.
17 Costa, K.S., Santos, S.L., Uzêda, R.S., Pinheiro, A.M., Almeida, M.A.O., Araújo,
18 F.R., McAllister, M.M., Gondim, L.F.P., 2008. Chickens (*Gallus domesticus*) are
19 natural intermediate hosts of *Neospora caninum*. Int. J. Parasitol. 38, 157–159.
20 Darwich, L., Cabezón, O., Echeverria, I., Pabón, M., Marco, I., Molina-López, R.,
21 Alarcia-Alejos, O., López-Gatius, F., Lavín, S., Almería, S., 2012. Presence of
22 *Toxoplasma gondii* and *Neospora caninum* DNA in the brain of wild birds. Vet.
23 Parasitol. 183, 377–381.
24 de Oliveira, U.V., de Magalhães, V.C.S., Almeida, C.P., Santos, I.D.A., Mota, D.A.,
25 Macêdo, L.S., Silva, F.L., Carvalho, F.D.S., Wenceslau, A.A., Munhoz, A.D.,

1 2013. Quails are resistant to infection with *Neospora caninum* tachyzoites. Vet.
2 Parasitol. 198, 209–213.
3 Du, L., Yang, D., Zhai, T., Gong, P., Zhang, X., Li, J., 2015. Detection of *Neospora*
4 *caninum*-DNA in brain tissues from pigeons in Changchun, Jilin (China). Vet.
5 Parasitol. 214, 171–173.
6 Dubey, J.P., Barr, B.C., Barta, J.R., Bjerkås, I., Björkman, C., Blagburn, B.L.,
7 Bowman, D.D., Buxton, D., Ellis, J.T., Gottstein, B., Hemphill, a., Hill, D.E.,
8 Howe, D.K., Jenkins, M.C., Kobayashi, Y., Koudela, B., Marsh, a. E., Mattsson,
9 J.G., McAllister, M.M., Modrý, D., Omata, Y., Sibley, L.D., Speer, C. a., Trees, a.
10 J., Uggla, a., Upton, S.J., Williams, D.J.L., Lindsay, D.S., 2002. Redescription of
11 *Neospora caninum* and its differentiation from related coccidia. Int. J. Parasitol.
12 32, 929–946.
13 Dubey, J.P., Carpenter, J.L., Speer, C.A., Topper, M.J., Uggla, A., 1988a. Newly
14 recognized fatal protozoan disease of dogs. J. Am. Vet. Med. Assoc. 192, 1269–
15 1285.
16 Dubey, J.P., Hattel, A.L., Lindsay, D.S., Topper, M.J., 1988b. Neonatal *Neospora*
17 *caninum* infection in dogs: isolation of the causative agent and experimental
18 transmission. J. Am. Vet. Med. Assoc. 193, 1259–1263.
19 Dubey, J.P., Jenkins, M.C., Ferreira, L.R., Choudhary, S., Verma, S.K., Kwok,
20 O.C.H., Fetterer, R., Butler, E., Carstensen, M., 2014. Isolation of viable
21 *Neospora caninum* from brains of wild gray wolves (*Canis lupus*). Vet. Parasitol.
22 201, 150–153.
23 Dubey, J.P., Jenkins, M.C., Rajendran, C., Miska, K., Ferreira, L.R., Martins, J.,
24 Kwok, O.C.H., Choudhary, S., 2011. Gray wolf (*Canis lupus*) is a natural
25 definitive host for *Neospora caninum*. Vet. Parasitol. 181, 382–387.

1 Ferroglio, E., Pasino, M., Romano, A., Grande, D., 2007. Evidence of *Neospora*
2 *caninum* DNA in wild rodents 148, 346–349.
3 Fuehrer, H., Blöschl, I., Siehs, C., 2010. Detection of *Toxoplasma gondii*, *Neospora*
4 *caninum*, and *Encephalitozoon cuniculi* in the brains of common voles (*Microtus*
5 *arvalis*) and water voles (*Arvicola terrestris*) by gene amplification techniques in
6 western Austria (Vorarlberg). Parasitol. Res. 107, 469–473.
7 Furuta, P.I., Mineo, T.W.P., Carrasco, A.O.T., Godoy, G.S., Pinto, A.A., Machado,
8 R.Z., 2007. *Neospora caninum* infection in birds: experimental infections in
9 chicken and embryonated eggs. Parasitology 134, 1931–1939.
10 Gonçalves, I.N., Uzêda, R.S., Lacerda, G. a., Moreira, R.R.N., Araújo, F.R., Oliveira,
11 R.H.M., Corbellini, L.G., Gondim, L.F.P., 2012. Molecular frequency and isolation
12 of cyst-forming coccidia from free ranging chickens in Bahia State, Brazil. Vet.
13 Parasitol. 190, 74–79.
14 Gondim, L.F.P., Mcallister, M.M., Pitt, W.C., Zemlicka, D.E., 2004. Coyotes (*Canis*
15 *latrans*) are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 34, 159–161.
16 Gondim, L.S.Q., Abe-Sandes, K., Uzêda, R.S., Silva, M.S.A., Santos, S.L., Mota,
17 R.A., Vilela, S.M.O., Gondim, L.F.P., 2010. *Toxoplasma gondii* and *Neospora*
18 *caninum* in sparrows (*Passer domesticus*) in the Northeast of Brazil. Vet.
19 Parasitol. 168, 121–124.
20 Hughes, J.M., Thomasson, D., Craig, P.S., Georgin, S., Pickles, A., Hide, G., Stud,
21 F., 2008. *Neospora caninum*: Detection in wild rabbits and investigation of co-
22 infection with *Toxoplasma gondii* by PCR analysis. Exp. Parasitol. 120, 255–260.
23 King, J.S., Šlapeta, J., Jenkins, D.J., Al-Qassab, S.E., Ellis, J.T., Windsor, P.A.,
24 2010. Australian dingoes are definitive hosts of *Neospora caninum*. Int. J.
25 Parasitol. 40, 945–950.

1 Mansourian, M., Khodakaram-Tafti, A., Namavari, M., 2009. Histopathological and
2 clinical investigations in *Neospora caninum* experimentally infected broiler
3 chicken embryonated eggs. Vet. Parasitol. 166, 185–190.
4 Martins, J., Kwok, O.C.H., Dubey, J.P., 2011. Seroprevalence of *Neospora caninum*
5 in free-range chickens (*Gallus domesticus*) from the Americas. Vet. Parasitol.
6 182, 349–351.
7 McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R. a., McGuire, A.M.,
8 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 28, 1473–
9 1478.
10 McGuire, A.M., McAllister, M., Wills, R.A., Tranas, J.D., 1999. Experimental
11 inoculation of domestic pigeons (*Columbia livia*) and zebra finches (*Poephila*
12 *guttata*) with *Neospora caninum* tachyzoites. Int J Parasitol 29, 1525–1529.
13 Mineo, T.W.P., Carrasco, A.O.T., Marciano, J. A., Werther, K., Pinto, A. A., Machado,
14 R.Z., 2009. Pigeons (*Columba livia*) are a suitable experimental model for
15 *Neospora caninum* infection in birds. Vet. Parasitol. 159, 149–153.
16 Mineo, T.W.P., Carrasco, A.O.T., Raso, T.F., Werther, K., Pinto, A. A, Machado,
17 R.Z., 2011. Survey for natural *Neospora caninum* infection in wild and captive
18 birds. Vet. Parasitol. 182, 352–355.
19 Molina-López, R., Cabezón, O., Pabón, M., Darwich, L., Obón, E., Lopez-Gatius, F.,
20 Dubey, J.P., Almería, S., 2012. High seroprevalence of *Toxoplasma gondii* and
21 *Neospora caninum* in the Common raven (*Corvus corax*) in the Northeast of
22 Spain. Res. Vet. Sci. 93, 300–302.
23 Munhoz, A.D., do Amaral, T.F., Gonçalves, L.R., de Moraes, V.M., Machado, R.Z.,
24 2014. *Gallus gallus domesticus* are resistant to infection with *Neospora caninum*
25 tachyzoites of the NC-1 strain. Vet Parasitol 206, 123–128.

1 Otranto, D., Llazari, A., Testini, G., Traversa, D., Di Regalbono, A.F., Badan, M.,
2 Capelli, G., 2003. Seroprevalence and associated risk factors of neosporosis in
3 beef and dairy cattle in Italy. Vet. Parasitol. 118, 7–18.

4 Pena, H.F.J., Soares, R.M., Ragozo, A.M.A., Monteiro, R.M., 2007. Isolation and
5 molecular detection of *Neospora caninum* from naturally infected sheep from
6 Brazil. Vet. Parasitol. 147, 61–66.

7 Rodrigues, A.A.R., Gennari, S.M., Aguiar, D.M., Sreekumar, C., Hill, D.E., Miska,
8 K.B., Vianna, M.C.B., Dubey, J.P., 2004. Shedding of *Neospora caninum*
9 oocysts by dogs fed tissues from naturally infected water buffaloes (Bubalus
10 bubalis) from Brazil. Vet. Parasitol. 124, 139–150.

11 Salant, H., Mazuz, M.L., Savitsky, I., Nasereddin, A., Blinder, E., Baneth, G., 2015.
12 *Neospora caninum* in crows from Israel. Vet. Parasitol. 212, 375–378.

13 Tranas, J., Heinzen, R.A., Weiss, L.M., 1999. Serological Evidence of Human
14 Infection with the Protozoan *Neospora caninum*. Clin. Diagn. Lab. Immunol. 6,
15 765–767.

16 Truppel, J.H., Montiani-Ferreira, F., Lange, R.R., Vilani, R.G.D.O.D.C., Reifur, L.,
17 Boerger, W., da Costa-Ribeiro, M.C.V., Thomaz-Soccol, V., 2010. Detection of
18 *Neospora caninum* DNA in capybaras and phylogenetic analysis. Parasitol. Int.
19 59, 376–379.

20 Vianna, M.C.B., Sreekumar, C., Miska, K.B., Hill, D.E., Dubey, J.P., 2005. Isolation of
21 *Neospora caninum* from naturally infected white-tailed deer (*Odocoileus*
22 *virginianus*) 129, 253–257.

23

Table 1. Serological prevalence of *Neospora caninum* in birds from different countries.

Animal specie	Country	Seroprevalence rate (%)	Test	Cut off	Reference
Chicken (*Gallus domesticus)*	Brazil	12.5	IFAT	1:50	Costa et al., 2008
Chicken (*Gallus domesticus*	Mexico	18.5	IFAT	1:25	Martins et al., 2011
	USA	7.2			
	Costa Rica	39.5			
	Grenada	71.5			
	Guatemala	44.0			
	Nicaragua	83.6			
	Argentina	58.1			
	Brazil	34.3			
	Chile	62.3			
	Colombia	11.2			
	Guyana	38.7			
	Peru	18.0			
	Venezuela	21.7			
Chicken (*Gallus domesticus*	Brazil	17	IFAT	1:50	Gonçalves et al., 2012
Chicken (*Gallus domesticus*	Brazil	36.5	IFAT	1:50	Camillo et al., 2015
Black volture (*Coragyps atratus*)	Brazil	0	IFAT	1:20	Mineo et al., 2011
Rock dove (*Columba livia*)	Brazil	0			
Eared dove (*Zenaida auriculata*)	Brazil	0			
Southern caracara (*Caracara plancus*)	Brazil	0			
Great-billed seed-finch (*Oryzoborus maximiliani*)	Brazil	0			
Atlantic canary (*Serinus canaria*)	Brazil	0			
Toco toucan (*Ramphastos toco*)	Brazil	0			
Blue fronted Amazon parrot (*Amazona aestiva*)	Brazil	0			
Hyacinth macaw (*Anodorhynchus hyacinthinus*)	Brazil	0			
Lear's macaw (*Anodorhynchus leari*)	Brazil	0			
Blue-and-yellow macaw (*Ara ararauna*)	Brazil	0			
Red-and-green macaw (*Ara chloropterus*)	Brazil	0			
Budgerigar (*Melopsittacus undulatus*)	Brazil	0			

Striped owl (*Asio clamator)*	Brazil	0			
Barn owl (*Tyto alba*)	Brazil	0			
Greater rhea (*Rhea americana*)	Brazil	0			
Common ostrich (*Struthio camelus*)	Brazil	0			
Common rave (*Corvus corax*)	Spain	35.8	IFAT	1:50	Molina-López et al., 2011
Hooded crow (*Corvus cornix*)	Israel	17.9	MAT/IFAT	1:100/1:50	Salant et al., 2015
Western jackdaw (*Corvus monedula*)	Israel	20.0			
House crow (*Corvus splendens*)	Israel	0			

Table 2. Birds as natural intermediate hosts for *Neospora caninum* due to detection of the parasite using different methods from bird tissues.

Animal specie	Location	Method	Tissue	Reference
Blue fronted Amazon parrot (*Amazona aestiva*)	Brazil	IHC	Cervical musculature	Mineo et al., 2011
Red-and-green macaw (*Ara chloropterus*)	Brazil	IHC	Cloacae musculature	Mineo et al., 2011
Chickens (*Gallus domesticus*)	Brazil	PCR (Nc5)	Brain	Costa et al., 2008
Sparrow (*Passer domesticus*)	Brazil	PCR (ITS-1)	Brain/Heart	Gondim et al., 2010
Hooded crow (*Corvus cornix*)	Israel	PCR (Nc5)	Brain	Salant et al., 2015
Western jackdaw (*Corvus monedula*)	Israel	PCR (Nc5)	Brain	Salant et al., 2015
Magpies (*Pica pica*)	Spain	PCR (Nc5)	Brain	Darwich et al., 2012
Common buzzard (*Buteo buteo*)	Spain	PCR (Nc5)	Brain	Darwich et al., 2012

Table 3. Mammals as natural intermediate hosts for *Neospora caninum* due to detection of the parasite using different methods from animal tissues.

Animal species	Location	Method	Tissue	Reference
Cattle (*Bos taurus*)	Argentina	PCR (Nc5), mouse bioassay	Brain	Campero et al., 2015.
European bison (*Bison bonasus bonasus*)	Poland	Culture isolation	Blood	Bién et al., 2010
Field mouse (*Apodermus sylvaticus*)	Italy	PCR (Nc5)	Kidney and skeletal muscle	Ferroglio et al., 2007.
House mice (*Mus musculus*)	UK	PCR (Nc5)	Brain	Hughes et al., 2006.
Rats (*Rattus norvergicus*)	Taiwan	PCR (Nc5)	Brains	Huang et al., 2004.
Sheep (*Ovis aries*)	Brazil	Dogs bioassay/PCR (Nc5)	Brain	Pena et al., 2007.
White-tailed deer (*Odocoileus virginianus*)	USA	Mouse bioassay	Brain	Vianna et al., 2005.
Water buffaloes (*Bubalus bubalis*)	Brazil	Dogs/gerbils bioassay	Brain	Rodrigues et al., 2004.
Dogs (*Canis familiaris*)	Brazil	Gerbil bioassay	Brain	Gondim et al., 2001.
Capybaras (*Hydrochaeris hydrochaeris*)	Brazil	PCR (Nc5/ITS-1)	Lymph nodes, heart, liver and blood	Truppel et al., 2010.
Goat (*Capra hircus*)	Argentina	Mouse/gerbils bioassay	Fetal fluid and brain, placenta	Unzaga et al., 2014.
Hoary fox (*Pseudolopex vetulus*)	Brazil	PCR (Nc5)	Brain	Nascimento et al., 2015.
Axis fawn (*Axis axis*)	Argentina	PCR (Nc5) and mouse/gerbil bioassay	Brain	Basso et al., 2014.
European brown bear (*Ursus arctos*)	Slovakia	PCR (Nc5/ITS-1)	Liver and spleen	Bartley et al., 2013
Mink (*Neovision vision*)	UK	PCR (ITS-1)	Brain	Bartley et al., 2013
Fox (*Vulpes vulpes*)	UK	PCR (ITS-1)	Brain	Bartley et al., 2013
Polecat (*Mustela putorius*)	UK	PCR (ITS-1)	Brain	Bartley et al., 2013
Ferret (*Mustela furo*)	UK	PCR (ITS-1)	Brain	Bartley et al., 2013
Badger (*Meles meles*)	UK	PCR (ITS-1)	Brain, neck muscle, liver	Bartley et al., 2013

Fallow Deer (*Dama dama*)	Switzerland	IHC, HP, PCR (Nc5)	Spinal cord, brain	Soldati et al., 2004.
Blue foxes (*Alopex lagopus*)	China	PCR (Nc5), HP, IHC	Brain	Yu et al., 2009.
Red foxes (*Vulpes vulpes*)	Czech Republic	PCR (Nc5)	Brain	Hůrková, Modrý, 2009.
Lesser kudu (*Tragelaphus imberbis*)	Germany	PCR (Nc5)	Brain, lung, heart, liver and spleen	Peters et al., 2001.
Raccoon (*Procyon lotor*)	USA	IHC, PCR (Nc5)	Brain	Lemberger et al., 2005
Black-tailed fawn (*Odocoileus hemionus columbianus*)	USA	HP, IHC	Kidney, liver and lung	Woods et al., 1994.
Eld's deer (*Cervus eldi siamensis*)	France	IHC	Brain	Dubey et al., 1996.
Alpaca (*Vicugna pacos)*	Peru	IHC, PCR (ITS-1)	Brain	Serrano-Martínez et al., 2004.
Llamas (*Llama glama*)	Peru	IHC, PCR (ITS-1)	Brain	Serrano-Martínez et al., 2004.
White rhinoceros (*Cerathotherium simum*)	South Africa	IHC	Heart	Williams et al., 2002.
Vole (*Microtus arvalis*)	Austria	PCR (Nc5)	Brain	Fuehrer et al., 2010
Water vole (*Arvicola terrestris*)	Austria	PCR (Nc5)	Brain	Fuehrer et al., 2010
Wild rabbits (*Oryctolagus cuniculus*)	UK	PCR (Nc5)	Brain, heart and tongue	Hughes et al., 2008
Rock squirrel (*Spermophilus variegatus*)	Mexico	HP, IHC, PCR (ITS-1)	Liver, heart, spinal cord and brain	Medina-Esparza et al., 2013.
European Pine Marten (*Martes martes*)	Netherlands	HP, IHC	Hert, liver and lung	Van der Hage et al., 2002
Parma wallaby (*Macropus parma*)	Austria	HP, IHC, PCR (ITS-1)	Heart	Cronstedt-Fell et al., 2012

4 OBJETIVOS

4.1 OBJETIVO GERAL

- Avaliar a importância dos pombos (*Zenaida auriculata*) na epidemiologia do *Neospora caninum*.

4.1 OBJETIVOS ESPECÍFICOS

- Padronizar uma técnica de ELISA para a pesquisa de anticorpos anti-*N. caninum* em pombos *Z. auriculata.*
- Verificar a ocorrência de *N. caninum* em pombos (*Z. auriculata*) naturalmente infectados capturados no município de Londrina, Paraná.
- Avaliar por meio da infecção experimental o potencial dos pombos (*Z. auriculata*) de atuarem como hospedeiro intermediário do *N. caninum.*

5 ARTIGO PARA PUBLICAÇÃO I**

SURVEY OF *NEOSPORA CANINUM* IN EARED DOVES (*ZENAIDA AURICULATA*) IN SOUTHERN BRAZIL

**Artigo redigido sob as normas de publicação do periódico Veterinary Parasitology, disponível em: https://www.elsevier.com/journals/veterinary-parasitology/0304-4017?generatepdf=true Acesso: 13/02/2016

1 **Abstract**

2 Neosporosis is an infectious disease caused by *Neospora caninum*, a protozoan
3 parasite that has worldwide distribution and is responsible for enormous economic
4 losses in cattle. Birds are considered a good bioindicator of environmental
5 contamination, since they feed on the ground, being exposed to *N. caninum* oocysts.
6 The aim of this study was to determine the occurrence of antibodies against *N.*
7 *caninum* and to verify the presence parasite DNA in tissue from free-ranging eared
8 doves (*Zenaida auriculata*) from Southern Brazil. For this purpose, serum and brain
9 samples were collected from 249 doves for ELISA and PCR analysis respectively.
10 The prevalence of *N. caninum* antibodies in doves was 31.72% (79/249) and
11 detection of DNA was not observed in none of birds. This is the first report of
12 antibodies against *N. caninum* in doves *Z. auriculata,* what show us that these birds
13 had previously contact with the parasite but since no DNA was detected, more
14 studies should be performed to elucidate the real importance of doves in the
15 epidemiologic cycle of the *N. caninum.*

16

17 **Keywords:** *Neospora caninum; Zenaida auriculata;* serology*;* ELISA*;* PCR

18

19 **1. Introduction**

20

21 *Neospora caninum* is a coccidian parasite of animals that mainly affect cattle,
22 being considered as one of the major responsible for reproductive problems by
23 infectious agents in cattle worldwide (Reichel et al., 2013). Canids, including dogs
24 (*Canis familiaris*), Australian dingoes (*Canis lupus dingo*), coyotes (*Canis latrans*)
25 and gray wolf (*Canis lupus*) are definitive hosts for this parasite, however it can infect

1 different species of mammals and birds (Darwich et al., 2012; Dubey et al., 2011;
2 Gondim et al., 2004; King et al., 2010; McAllister et al., 1998). An association
3 between the presence of birds on dairy farms and reproductive problems associated
4 with *N. caninum* has been reported, suggesting that these animals could play an
5 important role in the epidemiology of this disease (Bartels et al., 1999; Ould-
6 Amrouche et al., 1999).

7 Birds of *Zenaida auriculata* species (Des Murus, 1847), commonly known as
8 eared doves, belong to Columbiformes order and occur from the Antilles to Tierra del
9 Fuego. These animals are considered sinantropic, since they are not domesticated
10 animals and live close to humans, mainly due to the high food supply, favorable
11 environmental conditions and absence of predator, leading to population growth out
12 of control, generating consequently agricultural losses, aesthetic damage to buildings
13 and problems on public health. Moreover, these birds are an important source of food
14 for humans in some regions of Brazil (Souza et al., 2007) and may contribute to
15 transmission of zoonotic diseases (Cano-Terriza et al., 2015; Cong et al., 2012; Li et
16 al., 2015)

17 The aim of the present work was to study *N. caninum* infection in free-ranging
18 eared doves from Southern Brazil.

19

20 **2. Material and methods**

21

22 2.1 Animals and study locations

23 Two hundred and forty-nine eared doves (*Z. auriculata*), including 114 males
24 and 135 females were trap captured between January 2010 and December 2011 in
25 three different region of Londrina (23°08’47” to 23°55’46”S/50°52’23” to 51°19’11”W)

Paraná state, Southern Brazil. Those animals were obtained from soybean seed plant (n= 140), campus of the State University of Londrina (n= 80) and a dairy cattle farm (n= 29). All experimental procedures were approved by Animal Ethics Committee of State University of Londrina (CEEA no. 70/08) and the Brazilian Institute of the Environment (IBAMA-SISBIO no. 16.428-1)

2.2 Sample collection

All doves were euthanized following the guidelines established by Nacional Council for Animal Experimental Control (CONCEA/Brazil). Blood collection was performed by cardiac puncture and the resultant serum samples were stored at -20°C until further analysis. Brain tissue from doves were collected aseptically and submitted to molecular detection of the parasite.

2.3 Serology

In order to detect antibodies IgG against *N. caninum,* an enzyme-linked immunosorbent assay test (ELISA) was developed based on previously studies (Paré et al., 1995). For the preparation of antigen, tachyzoites of NC-1 strain were growth in Vero cell supplemented with RPMI medium, bovine fetal serum and penicillin (10,000 U) and streptomycin (10mg/ml). Subsequently, the cells were scraped and passed through 27G needles. The resultant solution was centrifuged 10,000 g for 10 min and the pellet was mixed with glass beads through vortex three times of 10 minutes each. The solution was centrifuged again following the same conditions described before and the pellet was discarded. Antigen solution was submitted to protein concentration measure by using a commercial kit (Pierce™ BCA, Thermo Fisher, USA). The antigen was stored at -20° until performance of serological tests.

1 Previously tests were conducted to determine the optimal antigen
2 concentration and dilutions of serum and conjugate. Flat-bottom 96 well polystyrene
3 microtitration plates (Nunc-Immuno Plate, MaxiSorp, Denmark) were coated with
4 0.1ml of crude antigen (2.5μg/ml) diluted in 0.1M carbonate buffer (pH 9.6) and
5 incubated overnight at 4°C. Afterward, the plates were washed three times with PBS-
6 tween 20 (50mM tris,100mM sodium chloride, 0.05% tween 20 and pH 7.4) using an
7 automatic microplate washer (Immunowash 1575, Biorad, USA) followed by block of
8 non-specific immune sites with carbonate buffer plus 8% nonfat dry milk and
9 incubation of 1h at 37°C. Control and test sera samples were diluted 1:20 in PBS-
10 tween 20 plus 5% nonfat dry milk and 0.1ml of this mixture were added to the
11 microtiter plates in duplicate and incubated at 37°C for 1 h. After another thrice of
12 rinsing, 0.1 ml of peroxidase-labeled anti-chicken IgY antibody (A9046, Sigma-
13 Aldrich, USA), diluted 1:500 in PBS-tween 20 plus 5% nonfat dry milk, was added in
14 each well and incubated at 37°C for 1 h.

15 Plates were washed thrice and the peroxidase activity was revealed by
16 adding 0.1 ml of tetramethylbenzidine solution (TMB Single Solution, Invitrogen,
17 USA). The reaction was stopped by adding 0.05 ml of 1 N HCl and the optical density
18 (OD) was read at 450nm in an ELISA microplate reader (iMark Microplate
19 Absorbance Reader, Biorad, USA). The same positive and negative control sera
20 were included in all plates and a corrected OD value was calculated for each sample
21 as previously described by Garcia et al. (Garcia et al., 2006) to control plate-to-plate
22 variation. Serum samples were considered positive when $OD_{corrected}$> [OD mean
23 (from negative control sera) + 3SD (standard deviation from negative control sera
24 from all plates)].

25

1 2.4 DNA extraction and PCR

2 For each animal, 0.5 g of brain tissue was homogenized and DNA extraction 3 was performed using a commercial kit (PureLink Genomic DNA Mini Kit, Invitrogen, 4 USA) following the manufacturer's instructions. DNA samples were stored at -20°C 5 until molecular analysis.

6 PCR reactions were performed to detect *N. caninum* DNA in brain tissue 7 from birds. Nc5 gene was targeted as previously described by Müller et al. (1996) 8 with minor modification. PCR reaction contained 2µl of genomic DNA, 1x PCR buffer, 9 0.2mM of each dNTP, 2.5mM $MgCl_2$,1.25 U of Taq polymerase (Platinum Taq DNA 10 Polymerase, Invitrogen, USA), 0.8µM of each primer (Np21-plus and Np6plus), in a 11 final solution of 25µl. Reactions was carried out in an automatic DNA thermal cycler 12 (Veriti Thermal Cycler, Applied Biosystems, USA) following the conditions: 94°C for 5 13 min for initial denaturation, 35 cycles of 94°C for 30 seg, 63°C for 30 seg, 72°C for 1 14 min followed by a final extension step at 72°C for 7 min. Positive and negative 15 controls, constituted of DNA from tachyzoites of Nc-1 strain and ultra-pure water 16 respectively, were included in all analyses. PCR products were analyzed by 17 electrophoresis through a 1.5% agarose gel stained with Sybr Safe DNA Gel Stain 18 (Invitrogen, USA) and visualized under UV light. A 100 bp DNA ladder (Invitrogen, 19 USA) was used in all agarose gel.

20

21 2.5 Statistical analysis

22 All the variables were analyzed by means of the chi-square test or Fisher's 23 exact test, using the Epi Info software, version 6.04b (DEAN et al., 1994). P-values ≤ 24 0.05 were considered to be significant.

25

3. Results

Out of 249 samples analyzed, 79 (31.7%) were considered to be positive by ELISA test. Regarding the sex of the doves, there was no significant statistical difference ($p>0.05$), with 33.3% (38/114) of males positive and 30.3% (41/135) of females positive at the test. There was a statistically difference between the capture site ($p<0.05$), since positivity was higher between doves from the soybean seed plant (38.6%, 54/140), compared with those from the university campus (23.7%, 19/80) and the dairy cattle farm (20.7%, 6/29) (Table 1). Average optical density from positive samples was 0.80±0.26, while for negative sample was 0.18±0.12. Optical density obtained by ELISA test of each animal from different capture site is plotted in Fig. 1. Serum samples with optical density equal or higher than 0.433 were considered to be positive.

According by nested PCR for PCR for Nc5 gene, no specific DNA fragment for *N. caninum* was observed in none of brain samples.

4. Discussion

To the best of the author's knowledge, this is the first study that reports an occurrence of antibodies against *N. caninum* in doves of *Z. auriculata* species, where a seroprevalence of 31.72% was observed. A previously study with eared doves has already been done, but different from us, the authors used IFA for *N. caninum* antibody detection, however, none animal was positive (Mineo et al., 2011). However, there are some reports showing that birds present an immune response against *N. caninum* infection (Costa et al., 2008; Gondim et al., 2010; Martins et al.,

1 2011). In all previously studies, serological tests were performed using either IFAT or
2 MAT, however here, an ELISA test was optimized and used for the first time for
3 detection of antibodies against *N. caninum* in birds.

4 It was not observed statistical difference between the sex of birds and
5 seropositivity against *N. caninum.* Similar results were found in studies with *T. gondii*,
6 where no difference serology considering sex of doves (*Z. auriculata*) and pigeon
7 (*Columba livia*) was found (Barros et al., 2014; Tsai et al., 2006). Regarding the
8 capture site, there was a statistically significant difference with higher number of
9 positive animals captured in a soybean seed plant. Presence of dogs in some areas
10 could be a reason for this difference, however, doves are able to fly up to 117 km
11 daily from breed colonies (Bucher and Bocco, 2009), then, they can also be infected
12 in an area different from the capture site. Although small number of doves from dairy
13 cattle farm was analyzed, positive results were found, what could contribute to
14 increase of reproductive problems, since previously studies have shown that
15 presence of birds on dairy farm leads to higher risk for abortions associated to *N.*
16 *caninum* (Bartels et al., 1999).

17 Birds are used as a good bioindicator of environmental contamination since
18 they usually fed on the ground, being exposed to several pathogens, including *N.*
19 *caninum* oocysts. Studies performed before showed that birds, as chickens, dove,
20 pigeon among others, are source of infection of *T. gondii* (Barros et al., 2014; Pena
21 et al., 2013), *Cryptosporidium* spp. (Li et al., 2015) and *Salmonella* spp. (Cano-
22 Terriza et al., 2015).

23 Costa et al (2008) were pioneers in using serological test to study
24 immunological response against *N. caninum* in birds. Using chickens as a model and
25 by means of IFAT with a cut off of 50 they observed an overall seroprevalence of

12.5%, however, when they compared the origin of these animals, outdoor chickens had higher seropositive samples compared with indoor group, evidencing that animals that have access to different environmental conditions may be more exposed to this parasite. Afterwards, others studies with chickens were performed, with seroprevalence ranging from 5.7% to 83.6% (Camillo et al., 2015; Gonçalves et al., 2012; Martins et al., 2011).

Antibodies against *N. caninum* have also been described in other bird species worldwide (Mineo et al., 2011; Molina-López et al., 2012; Salant et al., 2015). Seroprevalence studies with the common raven (*Corvus corax*) from Spain showed that 35.8% were positive (Molina-López et al., 2012) while 16.4% of ravens (*C. cornix*, *C. monedula* and *C. splendes*) from Israel were positive (Salant et al., 2015). This difference in the serological prevalence may be due to the species of bird analyzed, geographical region studied and serological and cut off used. In our study, we used an ELISA test, which is more sensitive than IFAT, and could explain a higher prevalence in this study than reported by Mineo et al (2011) that used IFAT to evaluate the presence of antibodies against *N. caninum* in doves *Z. auriculata.*

Regarding to molecular diagnostic, no DNA amplification was observed in all samples tested. It shows that probably this species of doves do not bear a chronic infection of *N. caninum* with no development of cyst in brain tissues. Du et al. (2015) reported for the first time that pigeons can be the intermediate host for *N. caninum* since DNA from the parasite was observed in brain samples, although no species name from these pigeons was mentioned. Other studies have been reported DNA of *N. caninum* in brain samples from birds, including chickens (*Gallus domesticus*) (Costa et al., 2008), sparrow (*Passer domesticus*) (Abdoli et al., 2015; Gondim et al., 2010), hooded crow (*C. cornix*), western jackdaw (*C. monedula*) (Salant et al., 2015),

magpies (*Pica pica*) and common buzzard (*Buteo buteo*) (Darwich et al., 2012). McGuirre et al. (1999) observed positive PCR results in pigeons (*Columba livia*) inoculated with different doses of *N. caninum* tachyzoites, even for those who was seronegative after 6 weeks post inoculation, showing that PCR can detect chronic *N. caninum* infection. Mineo et al. (2011) have been detected *N. caninum* in tissue from wild and captive birds, since positive immunostaining of cysts tissue with anti-*N. caninum* polyclonal antibodies were observed in the cloacae musculature of a red-and-green macaw (*Ara chioropterus*) and in the cervical musculature of a blue-fronted Amazon parrots (*Amazona aestiva*). The same authors have also detected Apicomplexa-like tissue cysts in the pectoral muscle of a pigeon (*C. livia*) however immunohistochemistry was not able to detect *N. caninum* antigens.

5. Conclusions

The present study reported for the first time antibodies against *N. caninum* in doves *Z. auriculata* naturally infected. We conclude that doves have contact with *N. caninum* leading to an immunological response but molecular detection was not observed, indicating that species of bird may be not a good host for this parasite, however further studies should be performed in order to evaluate the real importance of this doves in the epidemiologic cycle of the disease.

References

Abdoli, A., Arbabi, M., Dalimi, A., Pirestani, M., 2015. Molecular detection of *Neospora caninum* in house sparrows (*Passer domesticus*) in Iran. Avian Pathol.

1 44, 319–322.
2 Barros, L.D. De, Taroda, A., Zulpo, D.L., Cunha, I.A.L. Da, Sammi, A.S., Cardim,
3 S.T., Miura, A.C., Su, C., Machado, R.Z., Vidotto, O., Garcia, J.L., 2014. Genetic
4 characterization of *Toxoplasma gondii* isolates from eared doves (*Zenaida*
5 *auriculata*) in Brazil. Rev. Bras. Parasitol. Vet. 23, 443–448.
6 Bartels, C.J., Wouda, W., Schukken, Y.H., 1999. Risk factors for *Neospora caninum*-
7 associated abortion storms in dairy herds in The Netherlands (1995 to 1997).
8 Theriogenology. 52, 247–57.
9 Bucher, E.H., Bocco, P.J., 2009. Reassening the importance of granivorous pigeons
10 as massive, long-distance seed dispersers. Ecology 90, 2321-2327.
11 Camillo, G., Cadore, G., Ferreira, M., Braünig, P., Maciel, J., Pivoto, F., Sangioni, L.,
12 Vogel, F., 2015. *Toxoplasma gondii* and *Neospora caninum* antibodies in
13 backyard chickens in Rio Grande do Sul, Brazil. Rev. Bras. Ciência Avícola 17,
14 263–265.
15 Cano-Terriza, D., Guerra, R., Lecollinet, S., Cerdà-Cuéllar, M., Cabezón, O., Almería,
16 S., García-Bocanegra, I., 2015. Epidemiological survey of zoonotic pathogens in
17 feral pigeons (*Columba livia* var. *domestica*) and sympatric zoo species in
18 Southern Spain. Comp. Immunol. Microbiol. Infect. Dis. 43, 22–27.
19 Cong, W., Huang, S.-Y., Zhou, D.-H., Xu, M.-J., Wu, S.-M., Yan, C., Zhao, Q., Song,
20 H.-Q., Zhu, X.-Q., 2012. First report of *Toxoplasma gondii* infection in market-
21 sold adult chickens, ducks and pigeons in northwest China. Parasit. Vectors 5,
22 110.
23 Costa, K.S., Santos, S.L., Uzêda, R.S., Pinheiro, A.M., Almeida, M.A.O., Araújo,
24 F.R., McAllister, M.M., Gondim, L.F.P., 2008. Chickens (*Gallus domesticus*) are
25 natural intermediate hosts of *Neospora caninum*. Int. J. Parasitol. 38, 157–159.

1 Darwich, L., Cabezón, O., Echeverria, I., Pabón, M., Marco, I., Molina-López, R.,
2 Alarcia-Alejos, O., López-Gatius, F., Lavín, S., Almería, S., 2012. Presence of
3 *Toxoplasma gondii* and *Neospora caninum* DNA in the brain of wild birds. Vet.
4 Parasitol. 183, 377–381.
5 Du, L., Yang, D., Zhai, T., Gong, P., Zhang, X., Li, J., 2015. Detection of *Neospora*
6 *caninum*-DNA in brain tissues from pigeons in Changchun, Jilin (China). Vet.
7 Parasitol. 214, 171–173.
8 Dubey, J.P., Jenkins, M.C., Rajendran, C., Miska, K., Ferreira, L.R., Martins, J.,
9 Kwok, O.C.H., Choudhary, S., 2011. Gray wolf (*Canis lupus*) is a natural
10 definitive host for *Neospora caninum*. Vet. Parasitol. 181, 382–387.
11 Garcia, J.L., Navarro, I.T., Vidotto, O., Gennari, S.M., Machado, R.Z., da Luz Pereira,
12 A.B., Sinhorini, I.L., 2006. *Toxoplasma gondii*: comparison of a rhoptry-ELISA
13 with IFAT and MAT for antibody detection in sera of experimentally infected pigs.
14 Exp. Parasitol. 113, 100–105.
15 Gonçalves, I.N., Uzêda, R.S., Lacerda, G. A., Moreira, R.R.N., Araújo, F.R., Oliveira,
16 R.H.M., Corbellini, L.G., Gondim, L.F.P., 2012. Molecular frequency and isolation
17 of cyst-forming coccidia from free ranging chickens in Bahia State, Brazil. Vet.
18 Parasitol. 190, 74–79.
19 Gondim, L.F.P., Mcallister, M.M., Pitt, W.C., Zemlicka, D.E., 2004. Coyotes (*Canis*
20 *latrans*) are definitive hosts of Neospora caninum. Int. J. Parasitol. 34, 159–161.
21 Gondim, L.S.Q., Abe-Sandes, K., Uzêda, R.S., Silva, M.S.A., Santos, S.L., Mota,
22 R.A., Vilela, S.M.O., Gondim, L.F.P., 2010. *Toxoplasma gondii* and *Neospora*
23 *caninum* in sparrows (*Passer domesticus*) in the Northeast of Brazil. Vet.
24 Parasitol. 168, 121–124.
25 King, J.S., Šlapeta, J., Jenkins, D.J., Al-Qassab, S.E., Ellis, J.T., Windsor, P.A.,

1 2010. Australian dingoes are definitive hosts of *Neospora caninum*. Int. J.
2 Parasitol. 40, 945–950.
3 Li, J., Lin, X., Zhang, L., Qi, N., Liao, S., Lv, M., Wu, C., Sun, M., 2015. Molecular
4 characterization of *Cryptosporidium* spp. in domestic pigeons (*Columba livia*
5 *domestica*) in Guangdong Province, Southern China. Parasitol. Res. 114, 2237–
6 2241.
7 Martins, J., Kwok, O.C.H., Dubey, J.P., 2011. Seroprevalence of *Neospora caninum*
8 in free-range chickens (*Gallus domesticus*) from the Americas. Vet. Parasitol.
9 182, 349–351.
10 McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R. a., McGuire, A.M.,
11 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 28, 1473–
12 1478.
13 McGuire, A.M., McAllister, M., Wills, R.A., Tranas, J.D., 1999. Experimental
14 inoculation of domestic pigeons (*Columbia livia*) and zebra finches (*Poephila*
15 *guttata*) with *Neospora caninum* tachyzoites. Int. J. Parasitol. 29, 1525–1529.
16 Mineo, T.W.P., Carrasco, A.O.T., Raso, T.F., Werther, K., Pinto, A. A., Machado,
17 R.Z., 2011. Survey for natural *Neospora caninum* infection in wild and captive
18 birds. Vet. Parasitol. 182, 352–355.
19 Molina-López, R., Cabezón, O., Pabón, M., Darwich, L., Obón, E., Lopez-Gatius, F.,
20 Dubey, J.P., Almería, S., 2012. High seroprevalence of *Toxoplasma gondii* and
21 *Neospora caninum* in the Common raven (*Corvus corax*) in the Northeast of
22 Spain. Res. Vet. Sci. 93, 300–302.
23 Müller, N., Zimmermann, V., Hentrich, B., Gottstein, B., 1996. Diagnosis of *Neospora*
24 *caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization
25 immunoassay. J. Clin. Microbiol. 34, 2850–2852.

1 Ould-Amrouche, A., Klein, F., Osdoit, C., Mohammed, H.O., Touratier, A., Sanaa, M.,
2 Mialot, J.P., 1999. Estimation of *Neospora caninum* seroprevalence in dairy
3 cattle from Normandy, France. Vet. Res. 30, 531–538.
4 Paré, J.P., Hietala, S.K., Thurmond, M.C., 1995. An enzyme-linked immunosorbent
5 assay (ELISA) for serological diagnosis of *Neospora* sp. infection in cattle. J.
6 Vet. Diagn. Invest. 7, 352-359.
7 Pena, H.F.J., Vitaliano, S.N., Beltrame, M. A.V., Pereira, F.E.L., Gennari, S.M.,
8 Soares, R.M., 2013. PCR-RFLP genotyping of *Toxoplasma gondii* from chickens
9 from Espírito Santo state, Southeast region, Brazil: New genotypes and a new
10 SAG3 marker allele. Vet. Parasitol. 192, 111–117.
11 Reichel, M.P., Alejandra Ayanegui-Alcérreca, M., Gondim, L.F.P., Ellis, J.T., 2013.
12 What is the global economic impact of *Neospora caninu*m in cattle - the billion
13 dollar question. Int. J. Parasitol. 43, 133–142.
14 Salant, H., Mazuz, M.L., Savitsky, I., Nasereddin, A., Blinder, E., Baneth, G., 2015.
15 *Neospora caninum* in crows from Israel. Vet. Parasitol. 212, 375–378.
16 Souza, E.A,; Telino-Júnior, W.R., Nascimento, J.L.X.; Lyra-Neves, R.M., Azevedo
17 Júnior, S.M., Filho, C.L., Neto, A.S., 2007 Estimativas populacionais de
18 avoantes Zenaida auriculata (Aves Columbidae, DesMurs, 1847) em colônias
19 reprodutivas no Nordeste do Brasil. Ornithologia. 2, 28–33.
20 Tsai, Y.J., Chung, W.C., Lei, H.H., Wu, Y.I., 2006. Prevalence of antibodies to
21 *Toxoplasma* gondii in pigeons (*Columba livia*) in Taiwan. J. Parasitol. 92, 871.

Table 1. Association of sex and capture site with the presence of antibodies against *N. caninum* by an ELISA test in eared doves (*Zenaida auriculata*) from Southern Brazil.

Variables	Positive[a] (%)	Negative (%)	Total	p-value
Sex				
Male	38 (33.3)	76 (66.7)	114 (45.7)	0.71
Female	41 (30.3)	94 (69.7)	135 (54.3)	
Total	79 (31.7)	170 (68.3)	249 (100)	
Capture site				
Soybean seed plant	54 (38.6)	86 (61.4)	140 (56.2)	0.03
University campus	19 (23.7)	61 (76.3)	80 (32.1)	
Dairy cattle farm	6 (20.7)	23 (79.3)	29 (11.7)	
Total	79 (31.7)	170 (68.3)	249 (100)	

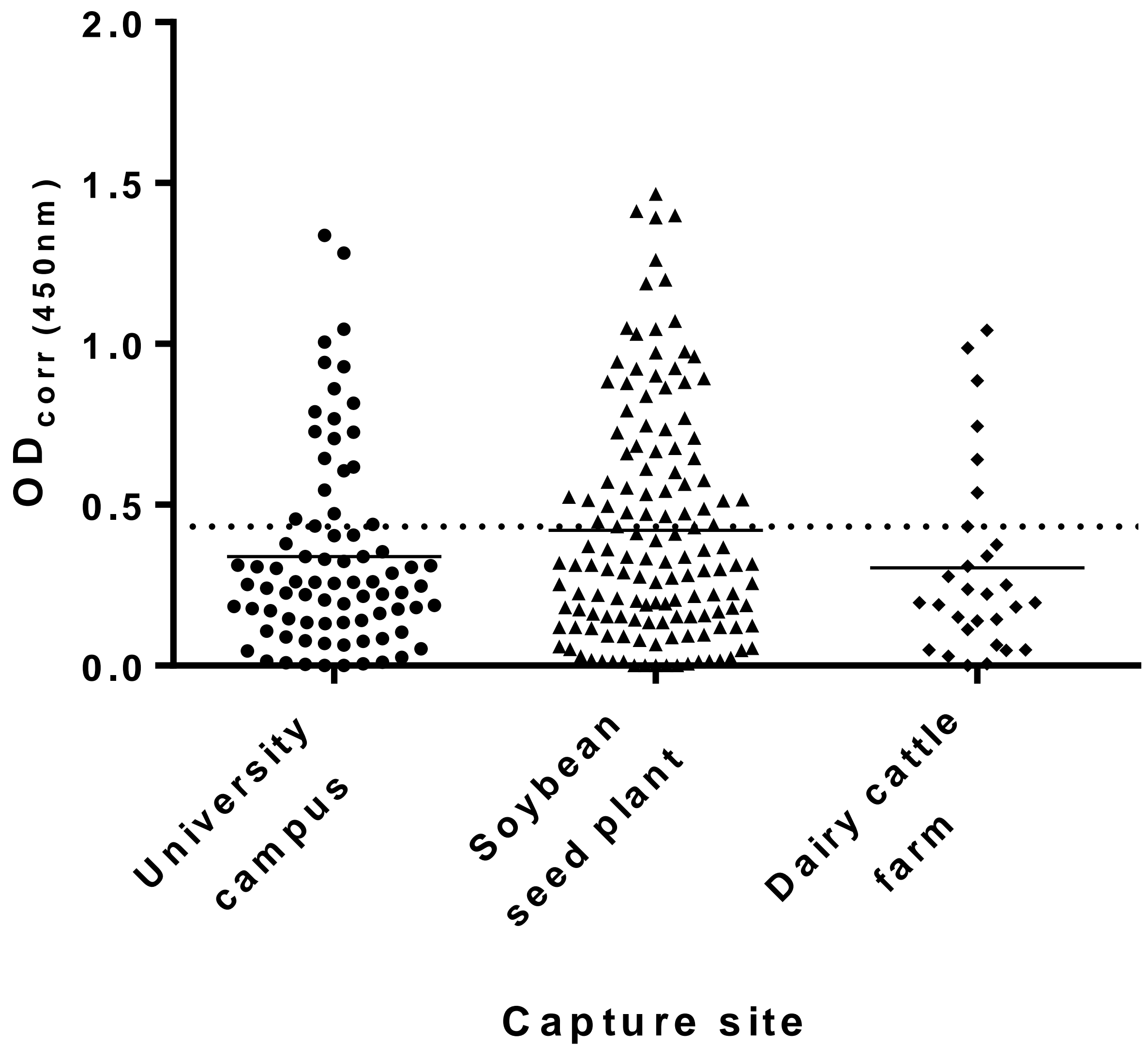


Fig. 1. Serological result by an ELISA test for *N. caninum* in eared doves (*Zenaida auriculata*) trap-captured from different location in Southern Brazil. Dashed line indicates the positive cut off (0.433) and line the mean.

1 **5 ARTIGO PARA PUBLICAÇÃO II****
2
3 **EXPERIMENTAL INOCULATION OF *NEOSPORA CANINUM* TACHYZOITES IN**
4 **EARED DOVES (*ZENAIDA AURICULATA*)**
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

** Artigo redigido sob as normas de publicação do periódico Experimental Parasitology, disponível em: https://www.elsevier.com/journals/experimental-parasitology/0014-4894?generatepdf=true Acesso: 13/02/2016

Abstract

Neospora caninum is an apicomplexan parasite with worldwide distribution. Although a positive association between the presence of birds and abortions in cattle associate to *N. caninum* has been reported, the role of the birds in the epidemiologic cycle of the parasite is unknown. Since there are no studies evaluating *N. caninum* in birds of *Zenaida auriculata* species, the present study aimed to determine if eared doves (*Z. auriculata*) can act as intermediate host for *N. caninum.* Eighteen birds were divided in four groups, G1, G2 and G3 and received $2x10^6$ tachyzoites of NC-1 strain by different routes, being subcutaneous, intramuscular and intraperitoneal respectively. G4 was composed by three animals and remained as negative control. Serum samples were collected weekly and one animal from G1, G2 and G3 were euthanized on the 7th and 14th day post inoculation (d.p.i.). The remaining birds were euthanized after 28 d.p.i.. After euthanasia and necropsy, tissues from the doves were submitted for histopathology, PCR and dog bioassay to detect the parasite. Dogs were fed with tissue from birds and monitored for 30 days. Serum samples were collected weekly for serological analysis and feces samples were collected daily until the end of the experiment for coproparasitological examinations. No dove showed clinical signs of infection, but all of them seroconverted after inoculation, with stronger immunological response in birds from G3. Positive PCR was found in lung tissue of one animal from G3 euthanized on the 7th d.p.i. and an inflammatory infiltrate was observed in the histopathology in the lung and kidney from these dove. No dogs shed oocysts or seroconverted. Our results indicate that intraperitoneal route induced infection in doves but the parasite may have been eliminated by the host, being doves resistant to chronic infection.

Keywords: *Neosporosis,* NC-1 strain, *Zenaida auriculata, birds,* dogs bioassay

1. Introduction

Neospora caninum is a protozoan parasite that was first described by Bjerkås et al (1984) in dogs who presented cysts associated with lesion in the central nervous system and skeletal muscles. Canids, including domestic dog, Australian dingo (*Canis familiaris*), coyote (*Canis latrans*) and gray wolf (*Canis lupus*) are definitive hosts of the parasite (Dubey et al., 2011; Gondim et al., 2004; King et al., 2010;

McAllister et al., 1998). Infection by *N. caninum* has been reported in many animals, including birds, however the zoonotic potential is unknown since just serological presence of antibodies against the parasite has been described in humans (Darwich et al., 2012; Dubey and Schares, 2011; Tranas et al., 1999).

Neosporosis is an important disease in cattle, being responsible for high economic losses worldwide due to reproductive problems such as abortion and persistently infected calves. In Brazil, economic losses are estimated in 153 million dollars per annum, mainly due to beef industry, which contributes to lower economic growth of the country (Reichel et al., 2013). Although the disease is important in dogs, since it can lead to neuromuscular problems, clinical cases are rare and isolated (Patitucci et al., 1997). In birds, neosporosis is not fully understood, however previously studies have demonstrated a positive correlation between the presence of birds and high seroprevalence in cattle, suggesting that birds could play an important role in the epidemiology of the disease in these animals (Bartels et al., 1999; Otranto et al., 2003).

The dove *Zenaida auriculata* is a bird from Columbiformes order, which includes other pigeon and dove species. These animals are native from Caribbean to South America, habiting mainly regions with semiarid climates, widely distributed throughout Brazilian territory (Souza et al., 2007). In some countries from South America, such as Argentina, Colombia and Brazil, the population of doves has increased out of control, being considered as a pest in the agriculture due to damage to crops (Bucher and Ranvaud, 2006). Although present during all year in some regions from Brazil, the population density of *Z. auriculata* is higher in the urban area, which contributes to damage to public environment and transmission of disease to human beings (Shibatta et al., 2009). Doves are also easily preyed upon by dogs, which could contribute to the life cycle of the parasite and disseminating of the disease.

The aim of the present study was to evaluate the infection of *N.* caninum in eared doves (*Z. auriculata*) and the potential of these birds to act as intermediate hosts of the parasite.

2. Material and methods

1 2.1 Animals
2
3 Eighteen eared doves (*Z. auriculata*), including 7 males and 11 females, trap
4 captured in Londrina city (23°08'47" to 23°55'46"S/50°52'23" to 51°19'11"W), Paraná
5 state, Southern Brazil, between September 2015 and October 2015 were used to
6 experimental study. All doves were tested to the presence of antibodies against *N.*
7 *caninum* by ELISA test and all were considered to be negative. Four mongrel dogs,
8 three female and one male, aged 5 months old and healthy were used to bioassay.
9 All dogs were seronegative to *T. gondii*, *Ehrlichia canis*, *N. caninum* and *Leishmania*
10 spp. The dogs were also vaccinated against canine distemper, adenovirus type 1 and
11 type 2, parainfluenza, parvovirosis, leptospirosis and rabies (Nobivac® Canine, MSD
12 Animal Health, USA) prior to experiment. The animals received anthelmintic
13 treatment with praziquantel, febantel, pyrantel pamoate and ivermectin (Canex
14 Premium, Ceva Santé Animale, Brazil), were fed with commercial feed, received
15 distilled water *ad libitum* and were allocated separately in individual stalls.
16 All procedures involving animals were approved by Animal Ethics Committee
17 of State University of Londrina (CEUA no. 157/15) and the Brazilian Institute of the
18 Environment (IBAMA-SISBIO no. 16.428-1)
19
20 2.2 *Neospora caninum* strain
21
22 *Neospora caninum* 1 (NC-1) strain (Dubey et al., 1988) were used to produce
23 the inoculum and for preparation of antigens for serological test. Tachyzoites were
24 grown in Vero cells supplemented with RPMI medium, bovine fetal serum and
25 penicillin (10,000 U) and streptomycin (10mg/ml). After five days of inoculation, most
26 of tachyzoites were free in the flask and cells were scraped and passed through 27G
27 needles. Then, the solution was centrifuged 2,000xg for 10 min and the supernatant
28 was discarded. The resultant pellet was resuspended in 1 ml of RPMI solution and
29 tachyzoites were counted using a Neubauer chamber.
30
31 2.3 Experimental design
32
33 The doves were divided into 4 groups, group 1 (G1, n=5), group 2 (G2, n=5),
34 group 3 (G3, n=5) and group 4 (G4, n=3). The G1, G2 and G3 received the same

1 inoculum concentration (2x10^6 tachyzoites) by different routes, being subcutaneous,
2 intramuscular and intraperitoneal respectively. The G4 was the negative control and
3 received PBS solution by intraperitoneal route.
4
5 2.4 Sample collection
6
7 Blood samples from the birds were collected by brachial venipuncture at days
8 0, 7, 14, 21 and 28 and serum samples obtained were stored at -20°C until
9 serological analysis. On day 7th and 14th, one animal of G1, G2 and G3 were
10 euthanized and tissue samples (brain, heart, liver, lung, kidney and pectoral muscle)
11 were collected for histopathological and PCR analysis. All other animals were
12 euthanized on day 28th and also had their tissue samples collected for histopathology
13 and molecular analysis. All animals were euthanized following the guidelines
14 established by the Nacional Council for Animal Experimental Control
15 (CONCEA/Brazil).
16
17 2.5 Enzyme-linked immunosorbent assay test (ELISA)
18
19 In order to detect antibodies IgG against *N. caninum*, an enzyme-linked
20 immunosorbent assay test (ELISA) was used. For the preparation of antigen,
21 tachyzoites of NC-1 strain were growth in Vero cell supplemented with RPMI
22 medium, bovine fetal serum and penicillin (10,000 U) and streptomycin (10mg/ml).
23 Subsequent, the cells were scraped and passed through 27G needles. The resultant
24 solution was centrifuged 10,000 g for 10 min and pellet was mixed with ceramic
25 beads through vortex three times of 10 minutes each. The solution was centrifuged
26 again following the same conditions described before and the pellet was discarded.
27 Antigen solution was submitted to protein concentration measure by using a
28 commercial kit (Pierce™ BCA, Thermo Fisher, USA). The antigen was stored at -20°
29 C until performance of serological tests.
30 Previously tests were conducted to determine the optimal antigen
31 concentration and dilutions of serum and conjugate. Flat-bottom 96 well polystyrene
32 microtitration plates (Nunc-Immuno Plate, MaxiSorp, Denmark) were coated with
33 0.1ml of crude antigen (2.5µg/ml) diluted in 0.1M carbonate buffer (pH 9.6) and
34 incubated overnight at 4°C. Afterward, the plates were washed three times with PBS-

tween 20 (50mM tris,100mM sodium chloride, 0.05% tween 20 and pH 7.4) using an automatic microplate washer (Immunowash 1575, Biorad, USA) followed by block of non-specific immune sites with carbonate buffer plus 8% nonfat dry milk and incubation of 1h at 37°C. Control and test sera samples were diluted 1:20 in PBS-tween 20 plus 5% nonfat dry milk and 0.1ml of this mixture were added to the microtiter plates in duplicate and incubated at 37°C for 1 h. After another thrice of rinsing, 0.1 ml of peroxidase-labeled anti-chicken IgY antibody (A9046, Sigma-Aldrich, USA), diluted 1:500 in PBS-tween 20 plus 5% nonfat dry milk, was added in each well and incubated at 37°C for 1 h.

Plates were washed thrice again and the peroxidase activity was revealed by adding 0.1 ml of tetramethylbenzidine solution (TMB Single Solution, Invitrogen, USA). The reaction was stopped by adding 0.05 ml of 1 N HCl and the optical density (OD) was read at 450nm in an ELISA microplate reader (iMark Microplate Absorbance Reader, Biorad, USA). The same positive and negative control sera were included in all plates and a corrected OD value was calculated for each sample as previously described by Garcia et al. (2006) to control plate-to-plate variation. Serum samples were considered positive when ODcorrected> [OD mean (from negative control sera) + 3SD (standard deviation from negative control sera from all plates)].

2.6 DNA extraction and PCR

A total of 0.25 mg of each tissue sample from each animal was submitted to DNA extraction using a commercial kit (PureLink Genomic DNA Mini Kit, Invitrogen, USA) following the manufacturer's instructions. DNA samples were stored at -20° C until molecular analysis.

PCR reactions were performed to detect *N. caninum* DNA in all tissue samples from birds collected during the necropsy. Nc5 gene was amplified as previously described by Müller et al. (1996) with minor modification, which amplifies a fragment of 337 bp. PCR reaction contained 2µl of genomic DNA, 1x PCR buffer, 0.2mM of each dNTP, 2.5mM MgCl2,1.25 U of Taq polymerase (Platinum Taq DNA Polymerase, Invitrogen, USA), 0.8µM of each primer (Np21-plus and Np6plus), in a final solution of 25µl. Reactions was carried out in an automatic DNA thermal cycler (Veriti Thermal Cycler, Applied Biosystems, USA) following the conditions: 94°C for 5

min for initial denaturation, 35 cycles of 94°C for 30 seg, 63°C for 30 seg, 72°C for 1 min followed by a final extension step at 72°C for 7 min. Positive and negative controls, constituted of DNA from tachyzoites of Nc-1 strain and ultra-pure water respectively, were included in all analyses. PCR products were analyzed by electrophoresis through a 1.5% agarose gel stained with Sybr Safe DNA Gel Stain (Invitrogen, USA) and visualized under UV light. A 100 bp DNA ladder (Invitrogen, USA) was used in all agarose gel.

2.7 Histopathology

All tissue samples collected during the necropsy of birds were kept in a 10% buffered formalin solution for 24 h, transferred subsequently to a 70% ethyl alcohol solution and embedded in paraffin. Paraffin-embedded tissues were serially sectioned at 4 µm thick and stained with hematoxylin-eosin (HE) for histopathological evaluation.

2.8 Bioassay in dogs

The dogs (n=4) were fed with a pool of tissue of approximately 70g (liver, lung, brain, heart, kidney and pectoral muscle) from inoculated birds. Each dog received a pool of all birds from different groups (i.e., dog 1 received tissue from all birds from G1, dog 2 birds from G2, dog 3 from G3 and dog 4 from G4). Physical examination of the dogs was performed every day during the experimental period. Blood samples from the dogs were collected by cephalic venipuncture at day 0 and every 7 days until the end of the experiment. Fecal samples were collected daily until 30th day after dogs were fed with tissue for coproparasitological examinations. During the experimental period, stalls were cleaned and disinfected every day with sodium hypochlorite at 2%.

2.9 Indirect fluorescent antibody test (IFAT)

Serum samples of all dogs were tested for antibodies IgG against *N. caninum* by an indirect fluorescent antibody test (IFAT) according with technique previously described (Dubey et al., 1988). Anti-dog IgG (F4012, Sigma-Aldrich, USA)

1 conjugated with fluorescein isothiocyanate were used as secondary antibodies.
2 Samples with titer ≥ 50 were considered positive.
3
4 2.10 Coproparasitological examinations
5
6 Fecal samples from dogs were examined daily during a period of 30 days by
7 sucrose flotation technique (density of 1.2 g/cm^3) for detection of *Neospora*-like
8 oocysts as previously described with minor modification (Sheather, 1923). The total
9 volume of feces from each day and each animal were homogenized and weighed.
10 Five grams from this homogenized sample were diluted in distilled water, filtered with
11 gauze and centrifuged at 1,200 g for 10 min. The pellet was mixed with sucrose
12 solution in a 15ml plastic tube and centrifuged at 1,200 g for 10 minutes. Afterwards,
13 25 µl from the upper meniscus of the solution was recovered and transferred to a
14 slide and covered with a coverslip for microscopic examination.
15
16
17 **3. Results**
18
19 All inoculated birds did not showed any clinical signs or died after infection.
20 The doves from all groups seroconverted after challenge, with a peak on 7 d.p.i.,
21 however OD decrease during the experiment period, being no detectable in one
22 animal inoculated through by subcutaneous and two by intramuscular route, while all
23 doves inoculated by intraperitoneal route were considered to be positive (Fig. 1). All
24 birds from control group remained negative through experiment. The OD mean of G3
25 (1.589±0.79) were higher than G1 (0.779±0.71) and G2 (0.723±0.52).
26 One dove from G3, euthanized 7 d.p.i., had lung tissue positive by means of
27 PCR. All of other samples were negative by molecular tests. In the histopathology
28 evaluation, an inflammatory focus of mononuclear leukocytes was observed in the
29 lung and kidney of the same bird that was positive in PCR, however no cyst
30 compatible with *N. caninum* was observed in all samples analyzed.
31 In the bioassay in dogs, no oocysts were detected in the dog feces during the
32 examination. Furthermore, none of the dogs showed any signs of clinical infection or
33 seroconversion after fed with tissue from inoculated birds. All results are summarized
34 in table 1.

4. Discussion

In the present study no eared dove showed signal clinical of infection or mortality, being considered as resistant to neosporosis. Previously studies have shown that other species from birds, such as quails (*Coturnix coturnix* japonica) (de Oliveira et al., 2013) and chickens (*Gallus gallus domesticus*) (Furuta et al., 2007; McGuire et al., 1999; Munhoz et al., 2014) are resistant to *N. caninum* infection by NC-1 tachyzoites, however Mineo et al. (2009) reported that pigeon from *Columba livia* species are susceptible, with dissemination of the parasite through lungs, heart, liver, spleen, kidney and central nervous system. This difference in susceptibility to *N. caninum* infection could be related to different biological models and difference in inoculum concentration, since they used a higher number (10^7) of parasites.

All doves had antibodies above the cut off seven days after inoculation, however, the OD mean from all group decreases during the experimental period. This is in agreement with studies performed by de Oliveira et al. (2013) and Mineo et al. (2009), who showed that birds inoculated with tachyzoites present a peak of antibodies usually 14 days post inoculation but rapidly decreases, being undetectable after 30 days by means of IFAT. Animals from G3, that received tachyzoites by intraperitoneal route, had higher OD in ELISA test. It corroborates previously studies that reported intraperitoneal inoculation of *N. caninum* tachyzoites induce stronger immune response compared mainly with subcutaneous inoculation (de Oliveira et al., 2013; McGuire et al., 1999; Mineo et al., 2009).

According with PCR analysis, only lung sample from one bird of G3 was positive. It seems that intraperitoneal inoculation is a better route to parasite dissemination in eared doves, what have been reported in pigeons (*C. livia*), where birds inoculated by intraperitoneal route had positive results in different techniques, as tissue culture, PCR and histopathology (McGuire et al., 1999). In our study, the dove that was positive in PCR was euthanized 7 days post inoculation, which shows that *N. caninum* disseminate during the first days after infection, being undetectable after 4 weeks, showing that eared doves are resistant to chronic infection with *N. caninum* tachyzoites, as reported in quails and chickens (de Oliveira et al., 2013; Furuta et al., 2007).

Only one dove showed alterations in the histopathology evaluation that could be associated with neosporosis, with presence of mononuclear inflammatory focus. A

mononuclear inflammatory infiltrate was also observed in livers from quails 3 and 7 d.p.i. with *N. caninum* tachyzoites. McGuire et al. (1999) observed typical lesions of neosporosis in one pigeon (*C. livia*) inoculated with higher dose of tachyzoites, although they used NC-2 and NC-Liverpool, different strains from the our study, what could explain this difference in the pathogenesis of chronic infection, since these alterations were observed in animals euthanized six weeks post inoculation. No lesions were observed in doves euthanized after 28 d.p.i. and similar results were observed by de Oliveira et al. (2013) and Munhoz et al. (2014), who did not find changes in histopathology of chronically infected birds.

In the bioassay, during all experimental period, no dogs showed clinical signs of infection, shed oocysts or seroconverted after fed with tissue from doves inoculated with *N. caninum.* Other studies have also reported that dogs fed with tissue from birds inoculated with NC1- strain did not shed oocysts or seroconverted (de Oliveira et al., 2013; Munhoz et al., 2014). These results suggest that *N. caninum* cysts were no present in tissues from doves and probably infection with NC-1 tachyzoites, different from other species, is not a good model to induce chronic infections in birds, as reported in quails and chickens (de Oliveira et al., 2013; Gondim et al., 2002; Munhoz et al., 2014).

5. Conclusions

Based on results presented in this study, we conclude that intraperitoneal route induced infection in doves (*Z. auriculata*), but these animals are not a good intermediate host for *N. caninum,* since it seems like the parasite may have been eliminated by the host, being considered resistant to chronic infection. Further studies, using oocysts as infection model are necessary to evaluate the course of infection and determine the importance of doves in the epidemiological cycle of neosporosis.

References

Bartels, C.J., Wouda, W., Schukken, Y.H., 1999. Risk factors for *Neospora caninum*-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). Theriogenology 52, 247–57.

1 Bjerkås, I., Mohn, S.F., Presthus, J., 1984. Unidentified cyst-forming sporozoon
2 causing encephalomyelitis and myositis in dogs. Z. Parasitenkd. 70, 271–274.
3 Bucher, E.H., Ranvaud, R.D., 2006. S31-4 Eared dove outbreaks in South America :
4 patterns and characteristics. Acta Zool. Sin. 52, 564–567.
5 Darwich, L., Cabezón, O., Echeverria, I., Pabón, M., Marco, I., Molina-López, R.,
6 Alarcia-Alejos, O., López-Gatius, F., Lavín, S., Almería, S., 2012. Presence of
7 *Toxoplasma gondii* and *Neospora caninum* DNA in the brain of wild birds. Vet.
8 Parasitol. 183, 377–381.
9 de Oliveira, U.V., de Magalhães, V.C.S., Almeida, C.P., Santos, I.D.A., Mota, D.A.,
10 Macêdo, L.S., Silva, F.L., Carvalho, F.D.S., Wenceslau, A.A., Munhoz, A.D.,
11 2013. Quails are resistant to infection with *Neospora caninum* tachyzoites. Vet.
12 Parasitol. 198, 209–213.
13 Dubey, J.P., Hattel, A.L., Lindsay, D.S., Topper, M.J., 1988. Neonatal *Neospora*
14 *caninum* infection in dogs: isolation of the causative agent and experimental
15 transmission. J. Am. Vet. Med. Assoc. 193, 1259–63.
16 Dubey, J.P., Jenkins, M.C., Rajendran, C., Miska, K., Ferreira, L.R., Martins, J.,
17 Kwok, O.C.H., Choudhary, S., 2011. Gray wolf (Canis lupus) is a natural
18 definitive host for *Neospora caninum*. Vet. Parasitol. 181, 382–387.
19 Dubey, J.P., Schares, G., 2011. Neosporosis in animals-The last five years. Vet.
20 Parasitol. 180, 90–108.
21 Furuta, P.I., Mineo, T.W.P., Carrasco, A.O.T., Godoy, G.S., Pinto, A.A., Machado,
22 R.Z., 2007. *Neospora caninum* infection in birds: experimental infections in
23 chicken and embryonated eggs. Parasitology 134, 1931–9.
24 Garcia, J.L., Navarro, I.T., Vidotto, O., Gennari, S.M., Machado, R.Z., da Luz Pereira,
25 A.B., Sinhorini, I.L., 2006. *Toxoplasma gondii*: comparison of a rhoptry-ELISA
26 with IFAT and MAT for antibody detection in sera of experimentally infected pigs.
27 Exp. Parasitol. 113, 100–5.
28 Gondim, L.F.P., Gao, L., McAllister, M.M., 2002. Improved Production of *Neospora*
29 *caninum* oocysts, cyclical oral transmission between dogs and cattle, and in vitro
30 isolation from oocysts. J. Parasitol. 88, 1159–1163.
31 Gondim, L.F.P., Mcallister, M.M., Pitt, W.C., Zemlicka, D.E., 2004. Coyotes (*Canis*
32 *latrans*) are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 34, 159–161.
33 King, J.S., Šlapeta, J., Jenkins, D.J., Al-Qassab, S.E., Ellis, J.T., Windsor, P.A.,
34 2010. Australian dingoes are definitive hosts of *Neospora caninum*. Int. J.

1 Parasitol. 40, 945–950.
2 McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R. a., McGuire, A.M.,
3 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 28, 1473–
4 1478.
5 McGuire, A.M., McAllister, M., Wills, R.A., Tranas, J.D., 1999. Experimental
6 inoculation of domestic pigeons (*Columbia livia*) and zebra finches (*Poephila*
7 *guttata*) with *Neospora caninum* tachyzoites. Int J Parasitol 29, 1525–1529.
8 Mineo, T.W.P., Carrasco, A.O.T., Marciano, J.A., Werther, K., Pinto, A.A., Machado,
9 R.Z., 2009. Pigeons (*Columba livia*) are a suitable experimental model for
10 *Neospora caninum* infection in birds. Vet. Parasitol. 159, 149–153.
11 Müller, N., Zimmermann, V., Hentrich, B., Gottstein, B., 1996. Diagnosis of *Neospora*
12 *caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization
13 immunoassay. J. Clin. Microbiol. 34, 2850–2.
14 Munhoz, A.D., do Amaral, T.F., Gonçalves, L.R., de Moraes, V.M., Machado, R.Z.,
15 2014. *Gallus gallus domesticus* are resistant to infection with *Neospora caninum*
16 tachyzoites of the NC-1 strain. Vet Parasitol 206, 123–128.
17 Otranto, D., Llazari, A., Testini, G., Traversa, D., Di Regalbono, A.F., Badan, M.,
18 Capelli, G., 2003. Seroprevalence and associated risk factors of neosporosis in
19 beef and dairy cattle in Italy. Vet. Parasitol. 118, 7–18.
20 Patitucci, A.N., Alley, M.R., Jones, B.R., Charleston, W.A., 1997. Protozoal
21 encephalomyelitis of dogs involving *Neospora caninum* and *Toxoplasma gondii*
22 in New Zealand. N. Z. Vet. J. 45, 231–5.
23 Reichel, M.P., Alejandra Ayanegui-Alcérreca, M., Gondim, L.F.P., Ellis, J.T., 2013.
24 What is the global economic impact of *Neospora caninum* in cattle - the billion
25 dollar question. Int. J. Parasitol. 43, 133–42.
26 Sheather A.L., 1923. The detection of intestinal protozoa and mange parasites by a
27 flotation technique. J. Comp. Pathology. 36, 266-275.
28 Shibatta, O.A., Galves, W., Carmo, W.P.D. do, Lima, I.P, de Lopes, E.V., Machado,
29 R.A., 2009. A fauna de vertebrados do campus da Universidade Estadual de
30 Londrina, região norte do estado do Paraná, Brasil. Semina. Ciências Biológicas
31 e da Saúde 30, 3–26.
32 Souza, E. a, Telino-Júnior, W.R., Nascimento, J.L.X., Lyra-Neves, R.M., Azevedo
33 Júnior, S.M., Filho, C.L., Neto, A.S., 2007. Estimativas populacionais de
34 avoantes *Zenaida auriculata* (Aves Columbidae, DesMurs, 1847) em colônias

1 reprodutivas no Nordeste do Brasil. Ornithologia 2, 28–33.
2 Tranas, J., Heinzen, R.A., Weiss, L.M., 1999. Serological evidence of human
3 infection with the protozoan *Neospora caninum.* Clin. Diagn. Lab. Immunol. 6,
4 765–767.

Table 1. Detection of *Neospora caninum* by different test in eared doves (*Zenaida auriculata*) inoculated with tachyzoites of NC-1 strain.

Group	**Dose**	**Via**[a]	**OD mean**[b]	**PCR**	**HP**[c]	**Bioassay in dogs**	
						Oocyst shedding	**IFAT titer**
G1	$2x10^6$	SC	0.779	0/5	-	-	0
G2	$2x10^6$	IM	0.723	0/5	-	-	0
G3	$2x10^6$	IP	1.589	1/5	+	-	0
G4	na	IP	0.146	0/5	-	-	0

n.a.: not applicable.
a: SC: subcutaneous; IM: intramuscular; IP: intraperitoneal
b: Optical density obtained by ELISA test
c: Histopathological examination

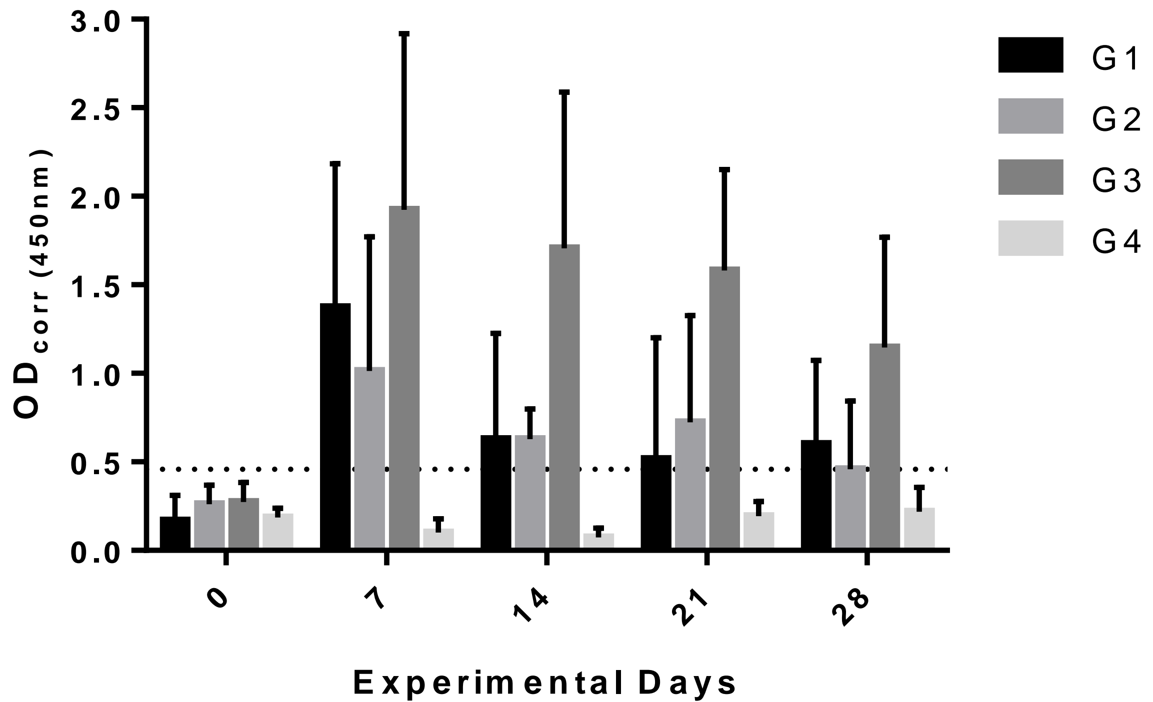


Fig 1. Immunological response of eared doves (*Zenaida auriculata*) inoculated with *Neospora caninum* tachyzoites evaluated by the indirect enzyme-linked immunosorbent assay (ELISA). All birds received the same parasite concentration by different routes, being G1 subcutaneous, G2 intramuscular and G3 intraperitoneal route (bars= OD mean from animals and error bars= standard deviation). Dashed line indicates positive cut off (OD=0.4579)

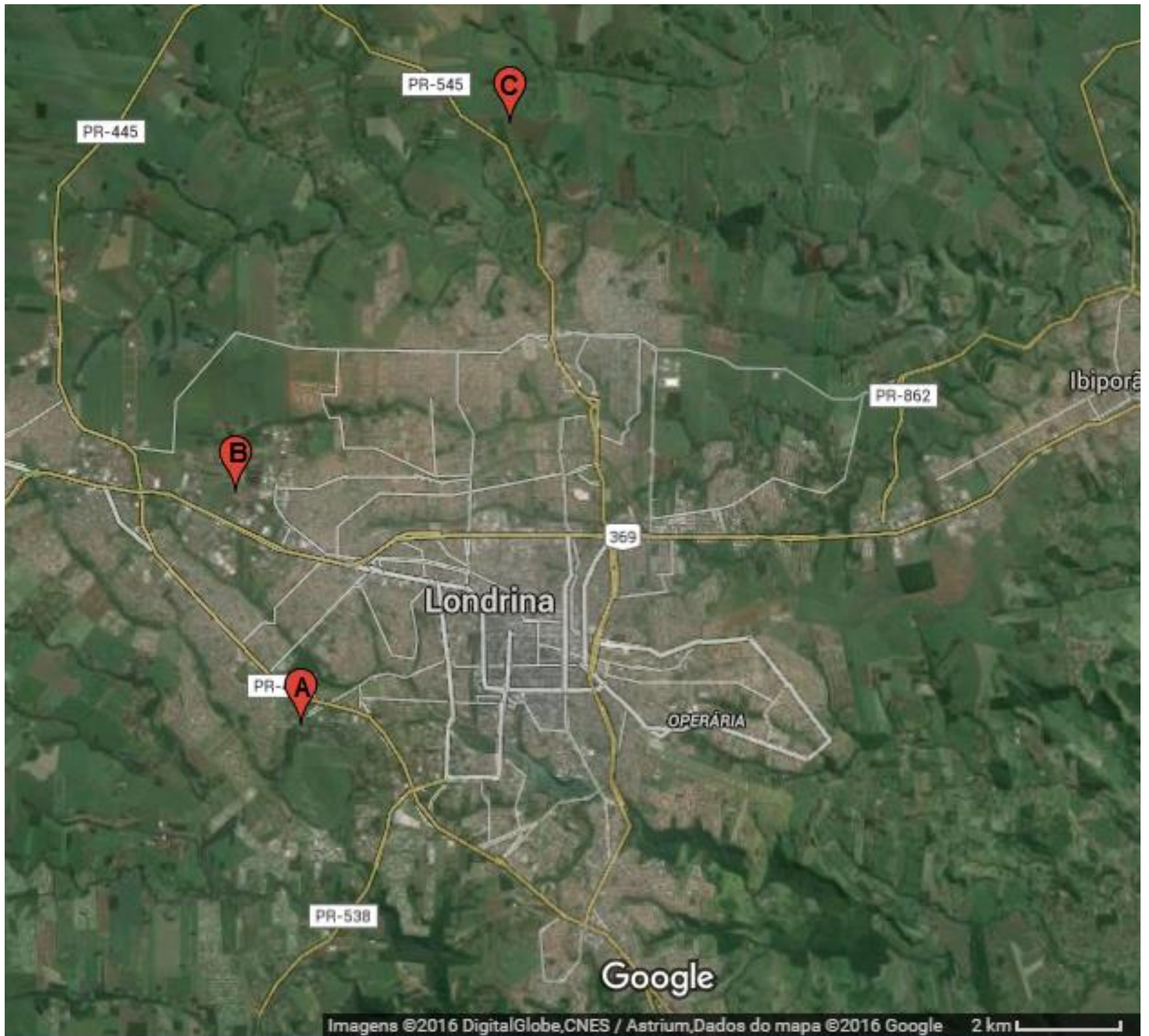
6 CONCLUSÃO

- Foi observado uma ocorrência de anticorpos contra *N. caninum* de 31.72%, em pombos da espécie Z. auriculata, sendo o primeiro relato da presença de anticorpos contra *N. caninum* nessa espécie de pombo.
- Pombos inoculados com taquizoítos da cepa NC-1 de *N. caninum* apresentaram uma elevada reposta imunológica 7 dias após inoculação
- Inoculação intraperitoneal de taquizoítos da cepa NC-1 de *N. caninum* foi capaz de induzir infecção em pombos, embora eles foram resistentes à infecção crônica.
- Cães alimentados com tecidos de pombos experimentalmente infectados com taquizoítos da cepa NC-1 não eliminaram oocistos ou soroconverteram, mostrando que os pombos da espécie *Z. auriculata* não são hospedeiros intermediários do *N. caninum* nas condições do presente estudo.

ANEXOS

ANEXO A

Mapa do local de captura dos pombos. A: Campus universitário; B:Indústria de processamento de grãos; C: Fazenda de bovinocultura leiteira.



ANEXO B

Ofício do Comitê de Ética em Experimentação Animal n° 70/2008

COMITÊ DE ÉTICA EM EXPERIMENTAÇÃO ANIMAL

OF. CIRC. CEEA Nº 70/2008 Londrina, 10 de setembro de 2008.

Prezado Pesquisador

O CEEA/UEL, reunido aos 09 de setembro do ano corrente, avaliou o projeto de pesquisa intitulado **"Caracterização genética de isolados de *Toxoplasma gondii* de pombas (*Zenaida auriculata*) do município de Londrina, Paraná"**, registrado no CEEA sob o nº 35/08, desenvolvido sob sua responsabilidade, julgando-o *aprovado* para execução por entender que os princípios éticos postulados pelo Colégio Brasileiro de Experimentação Animal estão respeitados.

Serão utilizados 384 pombos capturados em praças públicas do município de Londrina e 768 camundongos adultos procedentes do Biotério central da UEL.

Tendo em vista o grande número de pombos capturados e o ineditismo e a complexidade desse procedimento, o CEEA/UEL sugere que outras investigações relevantes sejam realizadas aproveitando material biológico colhido desses animais.

Cumpre orientar que caso se pretendam quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação do CEEA/UEL anteriormente à execução das modificações.

Sem mais para o momento, subscrevo-me.

Cordialmente,

Prof. Dr. Júlio Augusto Naylor Lisbôa
Coordenador do CEEA/UEL

Ilmo. Sr.
Prof. Dr. João Luis Garcia
Coordenador do Projeto
Departamento de Medicina Veterinária Preventiva
Centro de Ciências Agrárias

Campus Universitário: Rodovia Celso Garcia Cid (PR 445), Km 380 - Fone (43) 3371-4000 - PABX - Fax 3328-4440 - Caixa Postal 6001 - CEP 86051-990 - Internet http://www.uel.br
LONDRINA - PARANÁ - BRASIL

Form. Código 11.764 - Formato A4 (210x297)

ANEXO C

Ofício da Comissão de Ética no Uso de Animais n° 157/2015

Universidade Estadual de Londrina

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OF. CIRC. CEUA Nº 157/2015

Londrina, 04 de Agosto de 2015

Prezado Pesquisador,

A CEUA/UEL reunida em 07 de Julho de 2015 avaliou o projeto de pesquisa intitulado **"Avaliação da importância de pombos da espécie *Zenaida auriculata* na epidemiologia do *neospora caninum*"**, registrado sob o processo CEUA **n°8676.2015.81**, pesquisa do Centro de Ciências Agrárias, desenvolvido sob sua responsabilidade. Esclarecidos os aspectos metodológicos solicitados, o projeto está **aprovado** para execução entendendo-se que os princípios éticos postulados pelo Conselho Nacional de Controle de Experimentação Animal estão respeitados.

Serão utilizados 4 cães sem raça definida e com idade superior à 3 meses; e 24 aves *Zenaida auriculata*, sendo os cães animais abandonados ou doados e as aves capturadas na cidade e área rural por meio de armadilhas tipo arapuca. O projeto tem como objetivo avaliar a importância dos pombos (*Z. auriculata*) como hospedeiro intermediário do *Neospora caninum*. Para tanto, as aves serão divididas em quatro grupos experimentais, sendo um controle (que receberá uma dose de solução fisiológica i.m.) e os outros 3 desafiados com $1x10^7$ taquizoítos de *N. caninum* pela via subcutânea, intraperitoneal e intramuscular. Quarenta e cinco dias após o desafio, os animais serão eutanasiados por deslocamento cervical e os tecidos avaliados para a presença do parasita por meio da PCR, imunohistoquímica e bioensaio em cães. Os tecidos e demais materiais biológicos dos pombos serão fornecidos aos cães para alimentação para verificar a infectividade dos cistos teciduais e consequente eliminação dos oocistos do parasita por exames coproparasitológicos. As fezes serão coletadas diariamente durante 30 dias após a ingestão dos tecidos dos pombos e também será realizada coleta de sangue semanal. Os protocolos experimentais estão aprovados com previsão para execução em 14 meses.

Cumpre orientar que caso pretendam-se quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação da CEUA/UEL anteriormente à execução das modificações.

Coloco-me à disposição para quaisquer esclarecimentos que se fizerem necessária. Sem mais para o momento, subscrevo, cordialmente,

Waldiceu Ap. Verri Junior

Prof. Dr. Waldiceu Aparecido Verri Junior
Coordenador da CEUA/UEL

Ilmo. Sr.
Prof. Dr. João Luis Garcia
Coordenador do Projeto
Departamento de Medicina Veterinária Preventiva
Centro de Ciências Agrárias
Com cópia para André Junior da Conceição (Chefe da DP-IC/PROPPG), Chefe do Departamento de Medicina Veterinária Preventiva e Diretor(a) do Centro de Ciências Agrárias.

Campus Universitário: Rodovia Celso Garcia Cid (PR 445), km 380 - Fone (043) 3371-4000 PABX - Fax 3328-4440 - Caixa Postal 10.011 - CEP 86057-970 – Internet http://www.uel.br
LONDRINA - PARANA - BRASIL

ANEXO D

Autorização do Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA n° 16428-1

Ministério do Meio Ambiente - MMA
Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 16428-1	Data da Emissão: 14/07/2008 10:12	Data de Validade: 14/07/2009

Dados do titular

Registro no Ibama: 2509292	Nome: João Luis garcia	CPF: 605.644.319-15
Título do Projeto: Caracterização genética de isolados de Toxoplasma gondii de Pombas (Zenaida auriculata) do município de Londrina, Paraná.		
Nome da Instituição : Universidade Estadual de Londrina		CNPJ: 78.640.489/0001-53

Observações, ressalvas e condicionantes

1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
2	Esta autorização não exime o titular e a sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade.
3	Esta autorização não poderá ser utilizada para fins comerciais, industriais, esportivos ou para realização de atividades inerentes ao processo de licenciamento ambiental de empreendimentos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
4	A autorização para envio ao exterior de material biológico não consignado deverá ser requerida por meio do endereço eletrônico www.ibama.gov.br/cites. Em caso de material consignado, consulte www.ibama.gov.br/sisbio - menu Exportação.
5	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
6	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico.
7	Em caso de pesquisa em Unidade de Conservação Federal, o pesquisador titular deverá contactar a administração dessa unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infra-estrutura da unidade.

Locais onde as atividades de campo serão executadas

#	Município	UF	Descrição do local	Tipo
1	LONDRINA	PR	Universidade Estadual de Londrina	Fora de UC

Atividades X Táxons

#	Atividade	Táxons
1	Captura de animais silvestres in situ	Zenaida auriculata
2	Coleta/transporte de amostras biológicas in situ	Zenaida auriculata
3	Coleta/transporte de espécimes da fauna silvestre in situ	Zenaida auriculata (*Qtde: 384)

* Qtde. de indivíduos por espécie/localidade/unidade de conservação, a serem coletados durante um ano.

Material e métodos

1	Amostras biológicas (Aves)	Fezes, Ectoparasita, Sangue, Fragmento de tecido/órgão
2	Método de captura/coleta (Aves)	Outros métodos de captura/coleta(Arapucas-Armadas Isca)

Destino do material biológico coletado

#	Nome local destino	Tipo Destino
1	Universidade Estadual de Londrina	Pesquisa

Este documento (Autorização para atividades com finalidade científica) foi expedido com base na Instrução Normativa Ibama nº154/2007. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Ibama/Sisbio na internet (www.ibama.gov.br/sisbio).

Página 1/2

Código de autenticação: 83582191